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NAME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE W. H. VANDEN BORN

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THE UNIVERSITY OF ALBERTA

TRANSLOCATION OF GLYPHOSATE AND ^{14}C-ASSIMILATES IN CANADA THISTLE, LEAFY SPURGE AND TOADFLAX

by

© Ole Gottrup

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled

Translocation of Glyphosate and ^{14}C-Assimilates in Canada thistle, leafy spurge and toadflax

submitted by Ole Gottrup in partial fulfilment of the requirements for the degree of Master of Science in Weed Science.

.....
(Supervisor)

.....

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Date July , 1974

ABSTRACT

Field and greenhouse studies were concerned with the effects of glyphosate (N-phosphonomethylglycine) on Canada thistle (Cirsium arvense (L.) Scop.) and leafy spurge (Euphorbia esula L.).

Good control was obtained with foliarly-applied glyphosate (0.56 kg/ha) on Canada thistle in the greenhouse. The most lasting control was obtained after application at a mature growth stage of the plants. Glyphosate gave equally good control of Canada thistle in the greenhouse at 10, 21 and 27°C. In the field, good control was obtained after late fall application of 2.2 kg/ha on regrowth.

The recovery of total radioactivity from ^{14}C-glyphosate treated Canada thistle plants decreased with time after treatment. The amount of radioactivity present in the untreated plant parts increased with time up to one week. It then decreased towards the fourth week after treatment, thus indicating a loss of ^{14}C to the environment. Some of the activity was probably lost to the atmosphere as $^{14}CO_2$. No loss of radioactivity via the roots could be demonstrated. The activity from ^{14}C-glyphosate could be translocated to all secondary shoots. However, such shoots sometimes were bypassed. Glyphosate did not completely follow the assimilate stream in its translocation pattern.

There was good control of leafy spurge and Canada thistle grown in nutrient solution containing glyphosate. Foliar application of glyphosate at 4.56 kg/ha did not kill

leafy spurge in the greenhouse. Addition of surfactants to the commercial glyphosate formulation did not improve the control of leafy spurge.

Under high relative humidity the uptake and translocation of ^{14}C-glyphosate in leafy spurge was greatly enhanced.

Translocation of ^{14}C-assimilates in Canada thistle, leafy spurge and toadflax (Linaria vulgaris (L., Mill.) Hill) was studied under field conditions. The pattern of assimilate translocation in all three species was comparable. Some larger secondary shoots were bypassed while others imported ^{14}C-assimilates. In leafy spurge and toadflax, translocation of ^{14}C-assimilates from the main shoot sometimes took place to only a portion of the root.

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Table of Contents

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
1.1 Introduction	3
1.2 Canada thistle	4
1.3 Leafy spurge	6
1.4 Toadflax	8
1.5 Translocation of assimilates and herbicides	8
1.6 Glyphosate	10
MATERIALS AND METHODS	
2. Materials	13
2.1 Plant material and culture	13
2.2 Chemicals	14
3. General application of treatment	15
3.1 Greenhouse spray treatment	15
3.2 Drop treatment with unlabelled glyphosate (greenhouse)	15
3.3 Drop treatment with ^{14}C-labelled glyphosate (greenhouse and field)	15
3.4 Spray treatment on a single shoot (field)	15
3.5 $^{14}CO_2$ treatments	16
4. Experiments	17
4.1 Canada thistle in greenhouse experiments	17
4.1.1 Response to glyphosate	17
4.1.2 Translocation	18
4.1.3 Root abnormalities	18

Page

4.1.4 Translocation velocity 18

4.1.5 Influence of temperature 19

4.2 Canada thistle in field experiments 19

4.3 Leafy spurge 20

4.4 $^{14}CO_2$ field experiments with Canada thistle, leafy spurge and toadflax 21

4.5 Root uptake from culture solution 21

5. Visual injury evaluation 22

6. Sample preparation 23

6.1 Extraction of $^{14}CO_2$ treated plants 23

6.2 Extraction of ^{14}C-glyphosate treated plants 24

7. Autoradiography 24

RESULTS AND DISCUSSION

8. Canada thistle 26

8.1 Greenhouse experiments with unlabelled glyphosate 26

8.1.1 Effects of sublethal doses of glyphosate 26

8.1.2 Symptoms on shoots and leaves 30

8.1.3 Effects of sublethal doses of glyphosate on roots 30

8.1.4 Effects of glyphosate at three temperatures 34

8.1.5 Effects on secondary shoots along 45 cm long roots after glyphosate treatment of the end-shoot 40

8.1.6 Root uptake 41

8.2 Greenhouse experiment with ^{14}C-labelled glyphosate 45

Page

8.2.1 Recovery of ^{14}C from ^{14}C-glyphosate treated plants 45

8.2.2 Translocation velocity of ^{14}C to untreated plant parts after ^{14}C-glyphosate treatment 47

8.2.3 Root exudation 51

8.3 Field experiments with unlabelled glyphosate 52

8.3.1 Post-harvest application 52

8.4 Field experiments with ^{14}C-glyphosate 53

8.4.1 Recovery of ^{14}C from treated and untreated plant parts 54

8.4.2 Bypassing of secondary shoots 55

8.4.3 Distribution of ^{14}C in ^{14}C-glyphosate treated Canada thistle plants 55

8.4.4 Translocation of ^{14}C at four growth stages 59

9. Leafy spurge 64

9.1 Effects of treatments with unlabelled glyphosate 64

9.1.1 Symptoms on shoots and leaves 64

9.1.2 Control of leafy spurge 64

a. Spray treatments 64

b. Dipping 64

c. Addition of surfactants to the spray solution 67

9.1.3 Effects on secondary shoots along 30 cm long roots from glyphosate treatment of the end-shoot 69

9.1.4 Root uptake 69

9.2 Recovery of ^{14}C from ^{14}C-glyphosate treated plants 71

Page

10. Soil adsorption of ^{14}C-glyphosate 74

11. Translocation of $^{14}CO_2$ assimilates (field studies) 76

11.1 Canada thistle 76

11.2 Leafy spurge 82

11.3 Toadflax 87

SUMMARY AND CONCLUSIONS 92

LITERATURE CITED 95

LIST OF TABLES

Table		Page
1.	Number of new shoots set by Canada thistle at 10, 21 and 27°C after treatment with glyphosate at 0, 0.22 and 0.56 kg/ha	39
2.	Effect of temperature and glyphosate on dry weight of Canada thistle plants sprayed at the bud stage and harvested four weeks later	40
3.	Canada thistle shoots on 45 cm root sections measured and scored for injury symptoms after foliar treatment of the end-shoot with glyphosate	42
4.	Translocation of ^{14}C-assimilates in Canada thistle shoots on 45 cm root sections following treatment of the glyphosate treated end-shoot with $^{14}CO_2$. Same plants as in Table 3	43
5.	Effects of glyphosate on 25-day old Canada thistle plants transferred to nutrient solution containing the herbicide	45
6.	Effects of glyphosate on Canada thistle of increasing age, transferred to nutrient solution containing the herbicide	46
7.	Recovery of ^{14}C from Canada thistle plants, 20 cm tall, up to one week after treatment with ^{14}C-glyphosate	47
8.	Effect of post-harvest application of glyphosate on Canada thistle	53
9.	Recovery of ^{14}C from ^{14}C-glyphosate treated Canada thistle plants in the field	54
10.	Percentage of total ^{14}C recovered from different shoot categories and roots one week after foliar treatment of Canada thistle plants with ^{14}C-glyphosate in the field	56
11.	Distribution of radioactivity in four plant parts at four treatment stages and four harvest times	61

Table

		Page
12.	Control of leafy spurge with glyphosate	66
13.	Control of leafy spurge following dipping of the tallest shoot in glyphosate solution	67
14.	Leafy spurge control with glyphosate containing additional surfactant	68
15.	Leafy spurge evaluated for injury symptoms on each shoot after foliar treatment of one end-shoot with glyphosate	70
16.	Effects of glyphosate on leafy spurge grown in nutrient solution containing the herbicide	71
17.	Recovery of ^{14}C from leafy spurge treated with ^{14}C-glyphosate containing surfactant	73
18.	Distribution of ^{14}C-assimilates in Canada thistle 48 hours after treatment with $^{14}CO_2$	78
19.	Distribution of ^{14}C-assimilates in leafy spurge 48 hours after treatment with $^{14}CO_2$	83

LIST OF FIGURES

Figure		Page
1.	Equipment for $^{14}CO_2$ treatment in the field	16
2.	Effects of sublethal doses of glyphosate on Canada thistle at five growth stages	28
3.	Effects of sublethal doses of foliar applied glyphosate on a growing tip of Canada thistle	31
4.	Effects of sublethal doses of glyphosate on young leaves of Canada thistle grown in nutrient solution containing 10 ppm glyphosate	32
5.	Canada thistle roots photographed nine weeks after foliar spray with glyphosate at the C-8 stage (plants at bud-stage, cut down to 8 cm and sprayed)	33
6.	Transverse sections of Canada thistle roots showing the vascular cylinder of a normal root (A), and an enlarged vascular cylinder of a swollen root caused by glyphosate treatment (B)	35
7.	Enlargements of the sections in Figure 6	36
8.	Growth (in cm) of Canada thistle shoots at 10, 21 and 27°C, four weeks after treatment with glyphosate at 0, 0.22 and 0.56 kg/ha	38
9.	Distribution and recovery of ^{14}C in Canada thistle at different times after application of ^{14}C-glyphosate to four mature leaves of one shoot	48
10.	Distribution of ^{14}C in field grown Canada thistle plants at different times after application of ^{14}C-glyphosate	58
11.	Distribution of ^{14}C in Canada thistle one day after ^{14}C-glyphosate application	60
12.	Effects of sublethal doses of glyphosate on leafy spurge	65

Figures

		Page
13.	Soil adsorption of ^{14}C-glyphosate	75
14.	Distribution of ^{14}C-assimilates in Canada thistle plants 48 hours after application of $^{14}CO_2$ to a main shoot	79
15.	Distribution of ^{14}C-assimilates in leafy spurge plants 48 hours after application of $^{14}CO_2$ to a main shoot	84
16.	Distribution of ^{14}C-assimilates in toadflax plants 48 hours after application of $^{14}CO_2$ to a main shoot	88

INTRODUCTION

Every year farmers suffer severe losses because of weeds infesting their crops. Not only will the actual crop yield be reduced because of weed competition, but the quality of the crop also may be affected. It is desirable, therefore, from both an economical and an esthetic point of view to limit and, if possible, eliminate weeds from crop land.

Some weeds, especially deep-rooted perennials, are harder to control than others. Three such perennials, considered noxious weeds in western Canada, are Canada thistle, leafy spurge, and toadflax. These weeds are hard to control with herbicides commonly used on crop land. Herbicides effective via the roots are needed for effective contol.

A new postemergence non-selective herbicide, glyphosate, developed by Monsanto, was first released for experimental use in 1971. It effectively controls most annual and perennial weeds, but does not have the residual effects of soil sterilants (47). It also has very low toxicity to animals.

Glyphosate was chosen for these studies as it appeared to kill most herbaceous vegetation, including perennial weeds, without sterilising the soil. Seeding of a crop can take place shortly after application of the herbicide treatment.

The uptake, translocation, and effects of glyphosate were studied on Canada thistle and leafy spurge.

In addition, translocation of photosynthetic assimilates in Canada thistle, leafy spurge and toadflax was investigated under field conditions.

LITERATURE REVIEW

1.1 Introduction

One of the best methods for weed control is cultivation, but deep-rooted perennial weeds require repeated cultivation for complete control. The advent of herbicides revolutionized the control of these as well as many other weeds. Since the discovery of 2,4-D (2,4-dichlorophenoxyacetic acid) many new herbicides have been developed, and herbicides with different modes of action are now available for many uses.

The first herbicide to be used for control of noxious perennial weeds such as Canada thistle (Cirsium arvense (L.) Scop.), leafy spurge (Euphorbia esula L.) and toadflax (Linaria vulgaris (L., Mill.) Hill.) was 2,4-D and it is still the most widely used on Canada thistle (38). MCPA (4-chloro-o-tolyl-oxy-acetic acid) can be as effective as 2,4-D and is often used for Canada thistle control in oats and corn, since these crops are more tolerant to MCPA (38). But these two herbicides only kill the foliage without seriously damaging the roots. Other herbicides can be used to control the three perennial weeds mentioned, but these are less or non-selective. For example, 2,3,6-TBA (2,3,6-trichlorobenzoic acid) has little selectivity (38), while amitrole (3-amino-s-triazole) is non-selective (55). Picloram (4-amino-3,5,6-trichloropicolinic acid) and dicamba (3,6-dichloro-o-anisic acid) give good control of all three species, including their

underground parts. Grasses show some resistance to these two herbicides; however, they are too persistent in the soil for general weed control on cultivated land. Herbicide persistence is an undesirable environmental influence which is subject to growing public concern.

Ideally a herbicide should effectively control weeds without damaging the crop, it should be non-persistent, and non-toxic to animals. Glyphosate (N-phosphonomethyl glycine) appears to approach these ideals.

1.2 Canada thistle

Canada thistle is a perennial noxious weed found throughout Canada and the northern half of the United States (36, 38). It is native to Europe, western Asia, and northern Africa, and is believed to be introduced into Canada by early colonizers (31). Ten varieties or ecotypes were described by Hodgson (37). Since Canada thistle is abundant and very agressive it can rapidly infest cultivated areas, where it causes serious crop losses whenever a sizable infestation occurs. It becomes established readily in shelter belts, roadsides and other areas.

Canada thistle can spread by means of roots and seeds (36, 38). The extensive underground root system is most important in maintaining or increasing localized infestations. A well established plant is capable of infesting an area 7 m in diameter in one season (38). All underground parts are roots or vertical stems, and no

rhizomes exist (30, 63). While roots may be found to a depth of 6 to 7 m, with the main portion being 7 to 30 cm beneath the surface (34, 36, 62), shoots may emerge from a depth of 90 cm (29).

When investigating the reproductive ability of root fragments it was found that 5 mm fragments were unable to sprout, while 7 to 10 mm fragments sprouted readily (24, 29, 56). Shoots from 25 mm long root pieces emerged from a depth of 50 cm (29).

Canada thistle is a long day plant which requires a 16-hour photoperiod to flower. However, differences among ecotypes exist, as some varieties will flower under a 14-hour photoperiod at temperatures above 20°C (36, 41). The flowers may be perfectly dioecious (3, 32) or imperfectly dioecious (37), with the ratio of male to female plants being 1 to 3 (3).

Although seeds of Canada thistle have a low germination percentage (32), they are the principal means by which the plant is spread over long distances. A few of the viable seeds have practically no dormancy (3), while the majority need storage under natural conditions for three to six months to be able to germinate. Seeds may remain viable in the soil for up to 20 years (36). The seeds are considered to be relatively unimportant in establishing localized infestation (44). Man is foremost in introducing this plant to new areas (32).

To effectively control Canada thistle it is

essential that the root system be killed. Therefore, the degree of penetration and translocation of root-active foliarly applied herbicides is very important. The degree of penetration seems to depend on several factors, such as addition of surfactant, temperature, humidity, and wax deposition on the leaves. Translocation is reviewed in a later chapter.

Canada thistle can be controlled by herbicides such as amitrole, 2,3,6-TBA, atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), dicamba, and picloram (25, 26, 36) but these are not selective in most crops at the rates required. Temperature influences plant growth and response to herbicides. The optimum temperature for shoot growth of Canada thistle is 21°C (41). The effectiveness of 2,4-D (13, 39) dicamba and picloram for Canada thistle control is greater when the plant is growing at 21°C than at lower temperatures. The effectiveness is enhanced with increasing temperature up to 32°C.

Deposition of wax on the leaves will reduce the effect of foliarly applied herbicides, for example 2,4-D (35). The site of sampling, maturity of the plant, and ecotype influence the amount of wax present on the leaves (35).

1.3 Leafy spurge

Leafy spurge is a noxious perennial weed, native to Europe and Asia (64). It is becoming abundant in many localities in Canada (7). It is difficult to control

because of its extensive root system, its mode of reproduction, and its capacity to withstand adverse conditions (5, 14, 58, 64).

Roots of leafy spurge penetrate the soil to a depth of up to 305 cm, while shoots are capable of reaching the soil surface from roots at a depth of 90 cm (14, 15, 58). Root fragments from all depths down to 280 cm are capable of setting shoots if brought close to the soil surface. The regenerative capacity of roots is lowered when the plant is flowering (57).

Myers et al. (50) found all underground horizontal parts of leafy spurge to be roots. Two types are present, named long roots and short roots. The long roots which may grow vertically or horizontally make up the main framework of the root system. Buds arise on these in an acropetal manner (58). Additional buds can arise without any particular pattern. The short roots are produced by long roots; they are short-lived and do not give rise to buds (58).

Leafy spurge is difficult to control by chemical means, and no selective chemical control can be recommended at present. Picloram affords good control (69) but is unsuitable for crop use because of its persistence in the soil (70). A combination of 2,3,6-TBA prior to plowing and seeding of a corn crop, and spray of the crop with 2,4-D, can give good leafy spurge control, when repeated over two to three consecutive years (17).

1.4 Toadflax

Toadflax was introduced from Europe as a garden plant (7, 67) and is now an important weed in fields and waste places throughout the Canadian prairies (7, 67). It is a deep-rooted, vigorous, persistent perennial weed (4, 7, 67) which often establishes itself readily in sod, and crowds out legumes and most grasses (67).

Clear anatomical differences exist between Canada thistle and leafy spurge on the one hand and toadflax on the other. Toadflax does not possess a pericyclic meristem and, therefore, adventitious buds on the roots originate in the cortex (4) and are always associated with lateral roots.

Toadflax is more easily controlled than leafy spurge. It is susceptible to bromacil, monuron, and diuron used for total weed control (25). However it is quite resistant to terbacil (3-tert-butyl-5-chloro-6-methyluracil) (71), simazine (2-chloro-4,6-bis (ethylamino)-s-triazine), atrazine, and 2,4-D type herbicides (25, 67).

1.5 Translocation of assimilates and herbicides

It is generally accepted that foliarly applied phloem-mobile herbicides move together with assimilates in plants (1). Therefore, knowledge of the assimilate translocation patterns may be used to predict translocation patterns for such herbicides. But caution must be used, as Forde (23) showed for example, that studies of carbohydrate movement cannot be used to predict the movement of MH (1,2-dihydro-

3,6-pyridazinedione) in quack grass. If a plant is exposed to a $^{14}CO_2$- containing atmosphere, ^{14}C- assimilates will be formed in the plant and the movement of these can be monitored. It has been determined by this method that, at least in grasses and two species of Oxalis, young shoots and young roots are at first independent of each other, both feeding on reserves from the previous year (11, 51). In the next phase the shoots export assimilates to the active sinks of growing roots (11), and soon after to newly emerging shoots (38, 51). At a more mature stage the assimilates accumulate in the underground portions of the plant as food reserves (11, 38). Nyahoza et al. (51) concluded that in Poa pratensis (L.) the primary tillers and rhizome-tillers, like in other rhizomatous grasses, were independent, but the tiller-rhizome system could be re-integrated by defoliation of some of the tillers.

In Canada thistle, shoots emerging from roots feed on food reserves from these until the 5 cm stage, when they become selfsupporting with photosynthates. Soon after, when the shoot is approximately 15 days old, it begins to export carbohydrates through the roots to the active sinks of younger shoots (38, 49). This translocation of assimilates from shoots to growing roots and emerging shoots takes place until the early bud stage with no accumulation of food reserves in the roots. Thus, the carbohydrate content of the roots decreases and is lowest at the early bud stage (3, 34, 38). At this stage the rate of photosynthesis in the shoots is high, and carbohydrate content of the roots

increases rapidly (9, 36, 38) indicating a rapid downflow of assimilates (63). Active movement of assimilates takes place all season long, but the sink areas change (9).

Translocation of tracers including many herbicides takes place together with photosynthates in the phloem, from a source to a sink of assimilates (1, 28). Dicamba (9), picloram (65) and MCPA (49), for example, are readily translocated in the phloem in Canada thistle. MCPA moves with the assimilate stream in the perennial weeds Rumex crispus and Symphytum officinale (48, 49). Dicamba and picloram are also readily transported in the xylem (9, 65). However, maximal herbicidal movement does not always correspond with maximal transport of assimilates (28). For dicamba and picloram translocation in Canada thistle, no stage of growth was found optimal (9, 65). In the same species the translocation of MCPA decreased at the bud stage when the flow of assimilates is expected to be greatest, while in Rumex crispus and Symphytum officinale the accumulation of MCPA in the roots was greatest at the flowering stage (48, 49).

1.6 Glyphosate

Glyphosate (trade name: Roundup) is a postemergence herbicide, first released for experimental purposes in 1971. It has a relatively low mammalian toxicity (LD_{50} = 4320 mg/kg for rats) and little or no residual effects in the soil (47). Lack of residual effect was demonstrated by Baird (2). He seeded sensitive crops (soybean and corn) immediately after spraying with 71.6 kg/ha glyphosate. No

injury symptoms were found at any stage in either crop. Valgardson (68) reported some residual effects on seedlings of rape (Brassica campestris L.) seeded four days following soil application of 6.7 and 8.9 kg/ha of glyphosate. The partial to complete lack of residual effects is likely due to adsorption of glyphosate by soil constituents (46, 66) and possibly rapid breakdown (61, 66). Addition of one percent montmorillonite to the spray solution reduced the effect of the herbicide by 80 to 90 percent, thus indicating strong adsorption. However, Rieck et al. (61) state that adsorption cannot account for all of the reduction in herbicidal effect.

The possible mode of action of glyphosate in Lemna gibba and Rhizobium japonicum has been investigated by Jaworski (42). He discovered that growth inhibition caused by glyphosate could be alleviated by the addition of L-phenyl alanine and/or L-tyrosine to the nutrient medium. He suggested, therefore, that glyphosate inhibits the aromatic amino acid biosynthetic pathway. Glyphosate at 100 ppm mixed in agar also inhibits the growth of Fusarium fungi (46).

In 1972 and more so in 1973 this herbicide has been tested on a great variety of weeds and crops. Results of field research carried out by contributors to the Research Report of the Canada Weed Committee (59, 60) indicate that most weeds as well as crops can be controlled by glyphosate when foliarly applied. Annuals are killed off, but rapid reinfestation from germinating seeds occurs, since glyphosate has very limited residual effects. A crop seeded immediately

after spraying will limit the reinfestation (59, 60).

Although glyphosate is non-selective it may be used as a directed postemergence spray for selective weed control in e.g. cotton (Gossypium hirsutum L.) and soybean (Glycine max (L.) Merr.) (22, 27, 52). Dalapon (2,2-dichloropropionic acid) can be replaced by glyphosate at a lower dose (20).

Glyphosate will effectively control many perennial weeds. Control lasting for a year or more has been obtained on quackgrass (Agropyron repens (L.) Beauv.) (68), Canada thistle (8, 10, 69) and toadflax (69). Leafy spurge is more resistant to this herbicide (10, 69). On all species mentioned the most lasting control occurred after application at a mature stage, although control of Canada thistle appears to be possible at all stages (45). Translocation of glyphosate to the subterranean structures of quackgrass is sufficient within a few days to prevent new tillers from sprouting (68).

Increase of the spray volume from the recommended 280 ℓ/ha to 560 ℓ/ha may result in better herbicide-plant contact and, therefore, a better herbicidal effect (19).

Attempts have been made to increase the effect of glyphosate by additions of surfactants (18, 72, 73). Surfactants (MON 0027 and a type not specified) reduced the rate of glyphosate needed to control purple nutsedge (Cyperus rotundus L.) by 5 to 50 percent, depending on environmental conditions (72, 73).

MATERIALS AND METHODS

Experiments were conducted in the greenhouse during 1973 and 1974 and in the field at the University farm at Ellerslie during the summer of 1973.

2. Materials

2.1 Plant material and culture

Canada thistle (Cirsium arvense (L.) Scop.) and leafy spurge (Euphorbia esula L.) plants were started from seed in 1972. A single plant selected from each species served as a stock plant for all future experiments. Canada thistle, identified according to Hodgson's key (37) resembled the FI variety, but had a silvery fine pubescence like the LW variety.

The stock plants were grown in 12.7 cm pots and 45 x 30 x 7.7 cm flats in soil in the greenhouse. The soil used throughout the greenhouse experiments was a 3:2:1 mixture (Malmo clay loam:peat:sand). Plants required for subsequent experiments were vegetatively propagated from the stock plants, to avoid genetic variability. Root cuttings 6 to 7 cm long were planted in 12.7 cm pots. Longer root sections (45 cm) were planted in 45 x 30 x 7.7 cm flats. Leafy spurge roots with one or more visible buds were selected for propagation, while Canada thistle roots were propagated without such selection. Canada thistle was grown in a growth chamber or in the greenhouse while leafy spurge was kept in the greenhouse only. The growth chamber was set to provide a 16-hour photoperiod with a light intensity at plant level

of 15,000 to 17,000 lux from a mixture of fluorescent and incandescent lamps. Relative humidity was maintained between 50 and 60 percent. Day and night temperature was 21°C unless otherwise specified. In the greenhouse a 16-hour photoperiod and a temperature of 19 to 21°C was maintained throughout the winter. Temperatures in excess of 35°C were occasionally experienced during the summer. Of the Canada thistle roots planted, 95 percent or more sprouted, while for leafy spurge the percentage was 50 to 70.

For field studies 30 cm root pieces of both species were obtained from the greenhouse and planted in Malmo clay loam soil at 1.2 m intervals with the surrounding area kept free from other weeds.

Attempts to propagate toadflax (Linaria vulgaris (L. Mill.) Hill.) from root fragments in the greenhouse were unsuccessful. Hence, toadflax clones for the field studies were collected around the Ellerslie farm and transplanted to the site of the experiments. Some genetic variability may have been present, therefore.

2.2 Chemicals

The herbicide used was glyphosate (N-phosphonomethylglycine) (Mon 2139). It was supplied by Monsanto Co. (St. Louis, Missouri) as the isopropylamine salt containing surfactant. Radioactive glyphosate (specific activity: 1.51 mCi/ mmole) was labelled on the carbon atom adjacent to the phosphorus. It was supplied as the parent acid (N-phosphonomethyl-glycine), and was converted to the mono-

isopropyl amine salt by the addition of isopropylamine in a 1:1 molecular ratio in distilled water.

$Ba^{14}CO_3$ (specific activity: 5 mCi/mmole) was purchased from New England Nuclear.

3. General application of treatments

Unless specified otherwise treatments described herein applied to all experiments.

3.1 Greenhouse spray treatments

Spraying of plants in greenhouse experiments was carried out in a spray cabinet using a travelling 800067 Tee-jet nozzle at 2.8 atmospheres pressure. The nozzle was 48 cm above the plants and the herbicide was applied in 254 ℓ/ha of water.

3.2 Drop treatment with unlabelled glyphosate (greenhouse)

Unlabelled glyphosate was applied to four leaves. Each leaf received one 10 µℓ droplet of a 5000 ppm solution.

3.3 Drop treatment with ^{14}C-labelled glyphosate (greenhouse and field)

Labelled glyphosate at a total dose of 0.1 µCi per plant was applied in 4 drops of 5 µℓ each. One drop was placed on the adaxial side of each of four leaves in both field and greenhouse experiments.

3.4 Spray treatment on a single shoot (field)

The main shoot of a plant was sprayed with unlabelled glyphosate by means of a bottle attached to a spray

head and an aerosol-type propellant container. The shoot to be treated was enclosed in a plastic box from which one side had been removed to facilitate the spraying operation. The remainder of the plant was also shielded by a plastic cover if wind conditions made this necessary.

3.5 $^{14}CO_2$ treatments

For studies on translocation of photosynthetic assimilates, plants were exposed to a $^{14}CO_2$ containing atmosphere for 10 minutes in a 38 x 38 x 76 cm plexiglass enclosure. The $^{14}CO_2$ atmosphere was prepared as follows: 2 mg of Ba $^{14}CO_3$ (specific activity: 5 mCi/mmole) containing 50 µCi was weighed in a 20 ml glass vial; 1 to 2 ml of lactic acid was added to the vial. The $^{14}CO_2$ evolved was pumped into the plexiglass enclosure and circulated by means of a 17.5 cm battery-operated fan.

Figure 1. Equipment for $^{14}CO_2$ treatment in the field.

4. Experiments

All experiments were designed as randomized complete blocks. Plants were selected for uniformity. Each replicate was one plant.

4.1 Canada thistle in greenhouse experiments

4.1.1 Response to glyphosate

In the greenhouse three-month old plants at the bud stage were randomly separated into five groups, each group representing one growth stage. Each growth stage received three glyphosate application rates (0, 0.22, and 0.56 kg/ha) and was replicated nine times. The five growth stages were:

1. Plants sprayed at bud stage, approximately 50 to 70 cm high, 13 weeks old.

For the four following stages the shoots were cut down to 8 cm and sprayed:

2. immediately (C-8), 13 weeks old,
3. after regrowth to 12 cm (C-12), 15 weeks old,
4. after regrowth to 21 cm (C-21), 21 weeks old,
5. after regrowth to bud stage (C-bud), 24 weeks old.

Plant height and the number of shoots were recorded at treatment time, and five, nine and thirteen weeks after glyphosate application. Assessment of injury symptoms was

carried out after the same time intervals.

4.1.2 Translocation

The translocation of glyphosate through 45 cm long Canada thistle roots with two or more shoots was studied on 6-week old plants in the greenhouse. Twenty plants, two per flat, having more than one shoot each, were used. One end-shoot was drop-treated with unlabelled glyphosate (see 3.2). After three weeks the degree of injury on untreated shoots was evaluated visually.

4.1.3 Root abnormalities

Transverse freehand sections were cut of the originally planted 45 cm long roots (see 4.1.2), and stained with neutral red. Photographs were taken under the microscope using Kodak Panatomic-X black and white film.

4.1.4 Translocation velocity

In order to determine the translocation velocity of glyphosate in Canada thistle, an experiment was carried out with two-month old plants in the growth chamber. The plants were drop-treated with ^{14}C-glyphosate (see 3.3) and harvested 6, 24 hours, 4 days, 1, 3, and 4 weeks following treatment. Each treatment was replicated six times. At the time of harvest each plant was separated into: 1. treated leaves, 2. remainder of the treated shoot, 3. secondary shoots, and 4. roots. Untreated plants were separated into shoots and roots. Samples were stored at -20°C until they were extracted for radioactivity determination.

4.1.5 Influence of temperature

The influence of temperature on the response of Canada thistle to glyphosate was studied. Three sets of plants (four replicates), ten weeks old and at the early bud stage, were placed in growth chambers at constant temperatures of $10^{\circ}C \pm \frac{1}{2}$, $21^{\circ}C \pm 1$, $27^{\circ}C \pm 1$. Following a one week equilibration period, plants were sprayed (see 3.1) with 0, 0.22, and 0.56 kg/ha of glyphosate. At treatment time and four weeks later plant heights and the number of leaves and shoots per plant were recorded. The plants were then scored for visible injury symptoms and fresh and dry weights were determined.

4.2 Canada thistle in field experiments

Translocation of glyphosate in Canada thistle was studied in the field. The tallest shoot of each plant was drop-treated with labelled glyphosate (see 3.3) at four growth stages:

1. 14 cm tall (14)
2. 22 cm tall (22)
3. early bud stage, 30 cm tall (bud)
4. cut down to ground level at the early bud stage and treated after 4 cm of regrowth had appeared (C-4).

Plants treated at the various stages were harvested 1 hour, 1 day, 1 and 4 weeks following treatment. There were five replicates, two of which were prepared for autoradiography and three for extraction of ^{14}C-compounds.

A duplicate experiment was carried out in which the treated shoot was sprayed with 1.1 kg/ha of unlabelled glyphosate (see 3.4) in addition to the drop treatment with ^{14}C-labelled herbicide.

4.3 Leafy spurge

Experiments to test the effectiveness of glyphosate in controlling leafy spurge under greenhouse conditions were carried out. Plants were sprayed (see 3.1) with 2.2 and 4.5 kg/ha glyphosate or dipped in 1000, 5000, 10,000, and 20,000 ppm glyphosate solutions for 30 seconds. The effect of adding 0.1 and 0.5 percent of two surfactants, Tween 20 (polyoxyethylene (20) sorbitan monolaurate) and Atplus 411F (information about composition not available) to the above spray treatments was also tested.

Translocation in 30 cm long roots with more than one shoot each was studied by drop treating (see 3.2) a shoot at the end of the root section.

The actual amount of glyphosate taken up by leafy spurge and translocated out of the treated leaves was investigated. Two-month old plants were treated on ten consecutive leaves with one 5 µl drop per leaf of a solution of ^{14}C-glyphosate, giving a total dose of 0.3 µCi per plant. To the solution was added 0.5 percent of surfactant Tween 20. The experiment was carried out at low relative humidity (RH) (normal greenhouse humidity), and at high RH, where each plant was covered with a plastic bag one week prior to treatment and throughout the experiment,

which was done in triplicate.

4.4 $^{14}CO_2$ field experiments with Canada thistle, leafy spurge, and toadflax

Translocation of ^{14}C-assimilates was studied under field conditions in Canada thistle, leafy spurge, and toadflax. One shoot per plant was treated in bright sunshine. Plants were harvested immediately and 48 hours after treatment. Canada thistle was treated at the same four stages (14, 22, bud and C-4) as previously mentioned under 4.2. Leafy spurge and toadflax were treated at three stages of the treated shoot, namely:

	leafy spurge	toadflax
1.	16 cm tall (16)	24 cm tall (24)
2.	22 cm tall (22)	33 cm tall (33)
3.	bud stage, 26 cm (bud)	bud stage, 37 cm (bud).

Of four replicates two were prepared for autoradiography and two for extraction of ^{14}C-compounds.

4.5 Root uptake from culture solution

Studies were conducted on root uptake of glyphosate in Canada thistle and leafy spurge growing in nutrient solution.

Root sections, 2 to 3 cm long, were planted in flats. After two weeks, when one or two leaves had emerged, the plants were transferred individually to 125 ml foil wrapped Erlenmeyer flasks, filled to capacity with Hoagland No. 2 nutrient solution (33), modified according to the following recipe:

Stock solution	ml/l
1M KNO_3	4.5
1M $Ca(NO_3)_2$	2.0
1M $MgSO_4$	1.0
1M $NH_4H_2PO_4$	0.5
micronutrients	0.5
FeDTPA (5000 ppm) (Fe)	0.08

The nutrient solution was replenished as needed. At the beginning of treatment, when the shoots were 3 weeks old, the nutrient solution was renewed and fresh nutrient solution containing 0,1, 10 and 100 ppm glyphosate was added. Initial experiments showed that these doses were sublethal. To study the effect of plant age on the degree of injury, Canada thistle plants were similarly treated when they were 4, 6, 10 and 12 weeks old. The 12 week old plants were at the bud stage.

To determine if root exudation occurred, two plants were grown together in the same flask. One plant was drop-treated (see 3.2) and the other one was examined for visible injury symptoms after various time intervals. A second set of plants was grown in nutrient solution and drop-treated with labelled glyphosate. Aliquots of the nutrient solution of 1 ml were taken after 0, 6, 12, 24, 36, 48 hours, 3, 4, 5, and 7 days for liquid scintillation counting.

5. Visual injury evaluation

Herbicidal effects on aerial plant parts were

assessed by visually scoring the injury symptoms. The scores are from 0 to 9, where 0 is no injury and 9 is complete kill of the scored plant part.

6. Sample preparation

Plants were harvested, put in plastic bags, rapidly frozen on dry ice, and stored in a freezer at -20°C until the labelled materials could be extracted. All the quantitative determinations of radioactivity were done in a LS-200B Beckman liquid scintillation spectrometer at room temperature. Samples were counted for fifty minutes each or until the counting error was one percent. Sample counts were corrected for efficiency by a channels ratio method.

6.1 Extraction of $^{14}CO_2$ treated plants

The determination of the total radioactivity present in $^{14}CO_2$ treated plants was carried out by determining the radioactivity present in both the ethanol soluble and ethanol insoluble plant extracts.

The samples were thawed out and ground in 95 percent ethanol in a Sorvall mixer for 3 minutes. The samples were filtered using a Buchner funnel and Whatman No. 1 filter paper. Of the filtrate, 0.5 ml aliquots were transferred to scintillation vials to which 15 ml of scintillation fluid was added. The scintillation fluid used was prepared by dissolving 120 g naphthalene, 6 g 2,5-diphenyloxazole (PPO), and 0.5 g 1,4-bis-2-(5-phenyloxazolyi)-

benzene (POPOP) in 1,4-dioxane to make up one litre.

The extracted tissue was dried at 50°C and ground in a Wiley mill to pass a screen containing 15.7 mesh/cm. A 20 mg sample was weighed in scintillation vials to which 15 ml of Aquasol [1] and 4 ml of distilled water were added. When shaken, the mixture formed a gel in which the ground tissue was dispersed. The vials were kept in darkness for 24 hours prior to counting.

6.2 Extraction of ^{14}C-glyphosate treated plants

Samples were thawed, dried at 50°C and ground in a Wiley mill to pass a screen containing 15.7 mesh/cm. The samples then were extracted twice with distilled water. For each extraction 0.5 ml of one percent antibiotics solution (sodium ethyl mercuri thiosalicylate (thimerosal)) and 0.5 ml of ammoniumcarbonate solution (0.2 molar) and a variable amount of distilled water were added, and the mixture was shaken for four hours. The samples were filtered after each extraction using a Buchner funnel and Whatman No. 1 filter paper. Aliquots of 4.5 ml were transferred to scintillation vials and 15 ml of Aquasol was added. When vials were shaken the mixture formed a gel. Scintillation fluid commonly used with water aliquots was unsuitable because of precipitate formation on addition of the sample.

7. Autoradiography

The autoradiographic investigations were carried

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1 Aquasol is a premixed scintillation fluid obtained from Nuclear Chicago.

out according to the methods described by Crafts et al. (16, 54). Plants were harvested, placed between paper towels and rapidly frozen on crushed dry ice. They were freeze-dried for six to eight weeks. The dried plants were humidified, mounted on cardboard, flattened, and then exposed to Kodak Blue Brand medical x-ray film. $^{14}CO_2$ treated plants were exposed for five days, while ^{14}C-glyphosate treated plants were exposed for eight weeks. The films were developed in Kodak Liquid X-ray Developer at 21°C for 2.5 minutes and fixed for 10 minutes or longer in Kodak fixer.

RESULTS AND DISCUSSION

8. Canada thistle

8.1 Greenhouse experiments with unlabelled glyphosate

8.1.1 Effects of sublethal doses of glyphosate

Preliminary experiments in the greenhouse showed that a dose of 0.78 kg/ha of glyphosate was lethal to Canada thistle. Lower, sublethal doses influenced Canada thistle differently at different growth stages (Fig. 2). The most severe injury symptoms appeared five weeks after spraying at all stages tested. After this the plants slowly began to recover. After 13 weeks only plants treated with 0.22 kg/ha at the C-8 stage had recovered completely, but the ranking order of the stages in Fig. 2A after five weeks had not changed significantly after 13 weeks. Best control was obtained at the stage before appearance of the first bud (C-21, Fig. 2A). Plants were controlled significantly better at the bud stage than at the remaining three stages, among which there was no significant difference. The latter result was surprising since plants at the C-8 stage, which had only six to ten old yellowing leaves after cutting, suffered as much as did plants at the C-12 and C-bud stages, which had many new shoots as a result of the cutting (36). These results suggest that senescent leaves are capable of taking up harmful amounts of glyphosate. The most lasting control was obtained after application of the herbicide to the more mature plants, which is in agreement with field studies by others (10, 69).

The most characteristic effects of sublethal doses of glyphosate on Canada thistle were production of many secondary shoots (Fig. 2B) and reduced top growth (Fig. 2C), as has been observed under field conditions (45). Glyphosate treatment induced the production of more secondary shoots than did cutting (Fig. 2B). Mature plants that were not cut did not produce many secondary shoots, and the glyphosate treatments did not change this behavior significantly. The greatest increase in shoot numbers occurred after treatment with 0.22 kg/ha at the C-21 stage. Plants at the C-8 stage were sprayed immediately after cutting, hence all new secondary shoots produced up to nine weeks after cutting and spraying were counted. At the other stages, sprayed after some regrowth, only the secondary shoots which emerged after spraying were counted. This accounts for the large number of new secondary shoots produced by plants at the C-8 stage, since the plants produced most of the secondary shoots within the first few weeks after cutting. The reduction in top growth was greatest for plants at the C-21 and the C-bud stages. The tops of these plants died off resulting in the negative growth values shown in Fig. 2C. For plants at the other stages the top growth was inhibited compared to the control.

The question arises then: "Why is the early bud stage the most susceptible"? Under field conditions this is the stage at which the roots contain the least food reserves (3, 34, 38). The photosynthetic rate is high and the downward flow of assimilates rapid (9, 36, 38, 63),

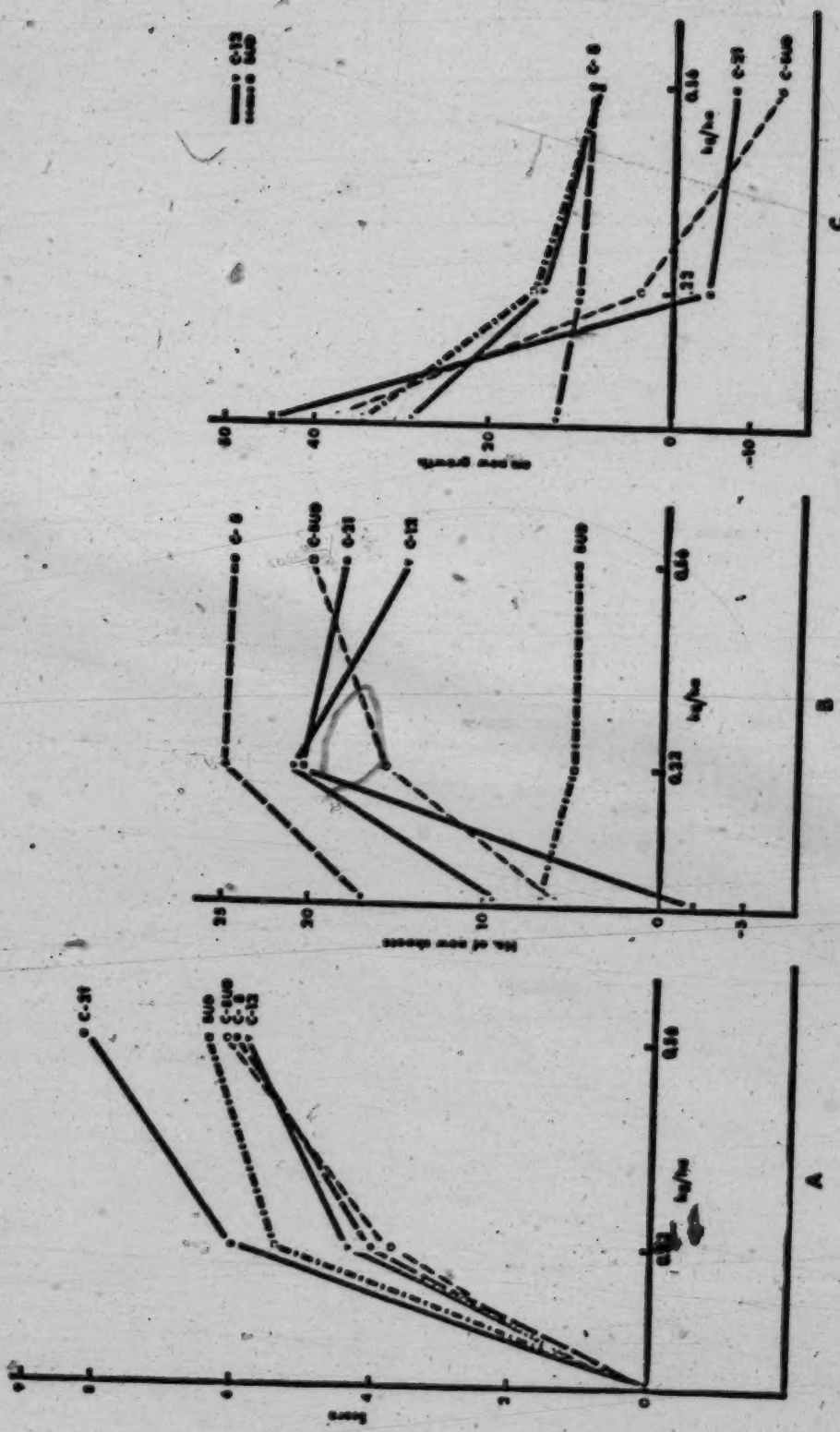
Figure 2. Effects of sublethal doses of glyphosate on Canada thistle at five growth stages.*

A. Injury scores five weeks after treatment, when symptoms were most severe. 0 = no injury, 9 = complete kill.

B. Number of new secondary shoots sprouted nine weeks after treatment.

C. Increase in height of tallest shoot nine weeks after treatment.

* The five growth stages were: bud stage (bud), cut to 8 cm and sprayed immediately (C-8), and sprayed after regrowth to 12 cm (C-12), 21 cm (C-21), or bud stage (C-bud).



causing increasing carbohydrate content in the roots. Assuming that glyphosate, like many other herbicides (1, 9, 28, 49, 65), moves with the assimilate stream, it would accumulate in the root most rapidly, and exert its phytotoxic effect most rapidly and completely, at the C-21 stage.

8.1.2 Symptoms on shoots and leaves

The first glyphosate symptoms appeared on young and emerging leaves (Figs. 3 and 4), beginning at the base. New leaves emerging after the glyphosate treatment were deformed, narrow and chlorotic (Fig. 3). The chlorosis was mainly in two longitudinal strips, one on each side of the midrib. The veins remained green. This effect was also observed after root uptake of glyphosate from nutrient solution (Fig. 4).

8.1.3 Effects of sublethal doses of glyphosate on roots

Foliar applied glyphosate at sublethal doses (0.22 and 0.56 kg/ha) caused swelling and reduction in number of Canada thistle roots nine weeks after spraying (Fig. 5). At this time the plants were beginning to recover from the treatment. The number of lateral roots visible on the swollen main root was severely reduced, but the root was still capable of producing new shoots. From the main root 5 cm sections containing shoot buds were replanted. These produced shoots free from visible glyphosate symptoms.

Figure 3. Effects of sublethal doses of foliar applied glyphosate (0.56 kg/ha) on a growing tip of Canada thistle. Sprayed at the bud stage and photographed eight weeks later.

Figure 4. Effects of sublethal doses of glyphosate on young leaves of Canada thistle grown in nutrient solution containing 10 ppm glyphosate. Treated when five weeks old and photographed two weeks later.

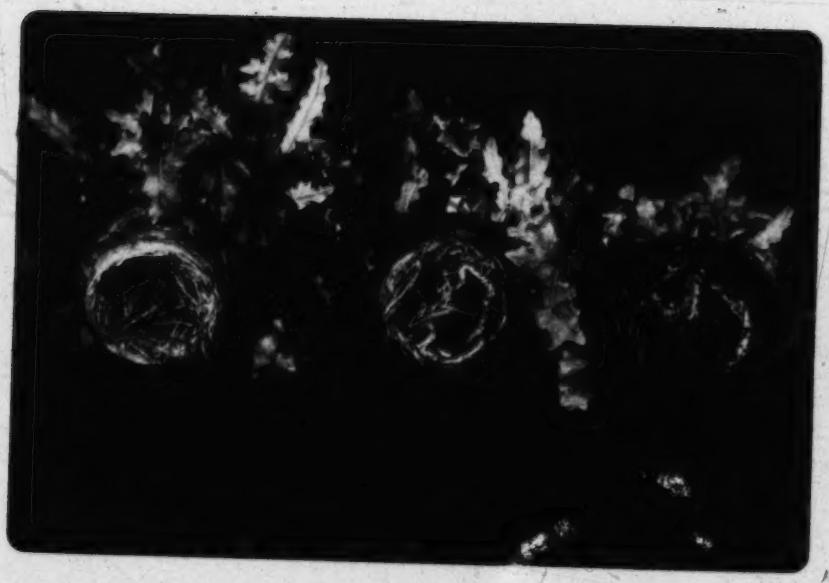


Figure 5. Canada thistle roots photographed nine weeks after foliar spray with glyphosate at the C-8 stage (plants at bud-stage, cut down to 8 cm and sprayed). Doses from left to right were 0, 0.22, and 0.56 kg/ha.

For further examination of the anatomy of these enlarged roots (Fig. 5), 45 cm long root sections were planted in flats in the greenhouse. When five weeks old they were treated with one 10 µl drop of a 5000 ppm glyphosate solution on each of four leaves. Eight weeks after treatment the planted root sections showed the same symptoms as the roots in Fig. 5. Transverse sections of the roots are shown in Figs. 6 and 7. The diameter has increased three to four times compared to the control. This is due to an increase in thickness of both the cortex and the vascular cylinder. Canada thistle roots (Fig. 6A) normally have two main parenchyma rays in the primary xylem with a number of narrower parenchyma rays. This arrangement of normal xylem was also found by Kreps et al. (43). In the treated root three main parenchyma rays, joined in the center, are apparent in the xylem (Fig. 6B). Several other rays cut partially through the xylem.

The number of xylem vessels per unit area in the abnormal root is much reduced when compared to the control (Fig. 7). The number of fibers in the xylem has increased. The phloem, as in the control, was located outside the xylem between the parenchyma rays. In an experiment described later it was demonstrated that these roots were capable of translocating ^{14}C-assimilates (Table 4, page 43).

8.1.4 Effects of glyphosate at three temperatures

The increase in height of Canada thistle was greatest at 21°C, reduced at 27°C and further reduced at

A

B

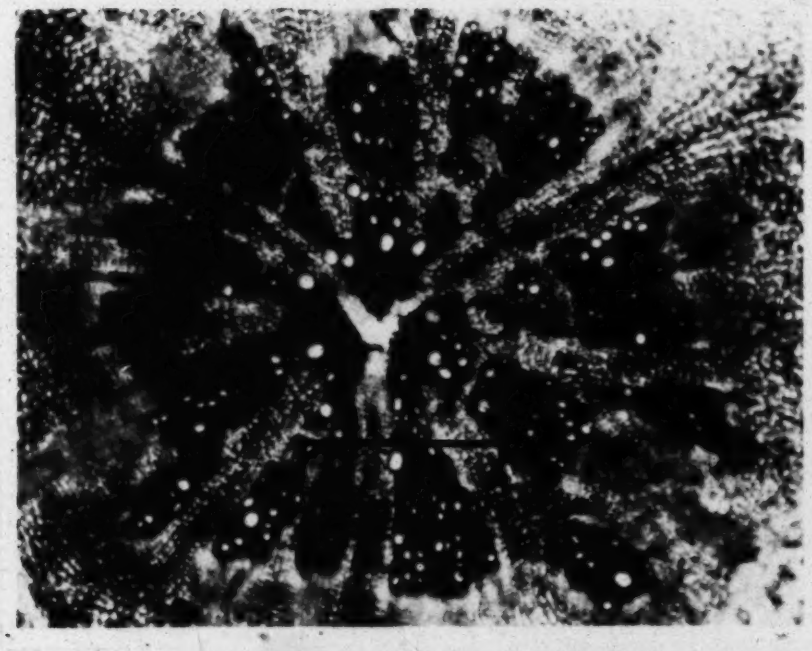
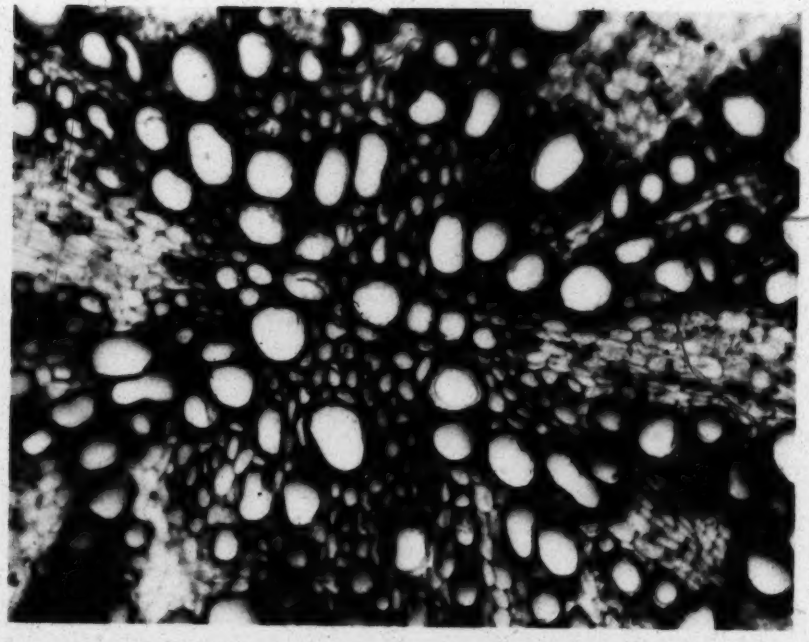


Figure 6. Transverse sections of Canada thistle roots showing the vascular cylinder of a normal root (A), and an enlarged vascular cylinder of a swollen root caused by glyphosate treatment (B) (x 30). Marked areas are enlarged in Fig. 7. Five-week old shoot treated with 4 x 10 µℓ of a 5000 ppm solution. Sectioned eight weeks later. Five replicates.



A

B

Figure 7. Enlargements of the framed sections in Fig. 6, from control (A) and glyphosate treated plant (B) (x 120).

10°C (Fig. 8). This is in agreement with the results of Hunter and Smith (41). Glyphosate effected a significant reduction in shoot height at the three temperatures and two rates tested. The greatest height reduction occurred with the 0.56 kg/ha rate at 21°C. At 10°C, when Canada thistle was not growing vigorously, the height reduction was also highly significant. In contrast to this the auxin-type herbicides 2,4-D, dicamba, and picloram do not provide as good growth control at low temperature (16°C) as at 21 and 27°C, when Canada thistle grows more vigorously (13, 39, 41).

The number of new secondary shoots increased considerably with increasing temperature from 21 to 27°C (Table 1). Glyphosate treatment, however, did not affect the number of new shoots produced. Similar results were found in an earlier experiment (Fig. 2), in which plants were grown at 21°C .

At 0.56 kg/ha of glyphosate visual injury symptoms observed at 27°C differed from those at 10 and 21°C. At 27°C the new secondary shoots were compact with narrow green leaves. At the lower temperatures (Fig. 3) leaves emerging after glyphosate treatment were composed of a midrib and a reduced blade area, present as a narrow band on either side of the midrib. At 10°C the plants did not show any glyphosate injury symptoms until the fourth week, when both sprayed and recently emerged shoots became chlorotic. Treated shoots with only slight chlorosis collapsed during the fourth week at 10°C.

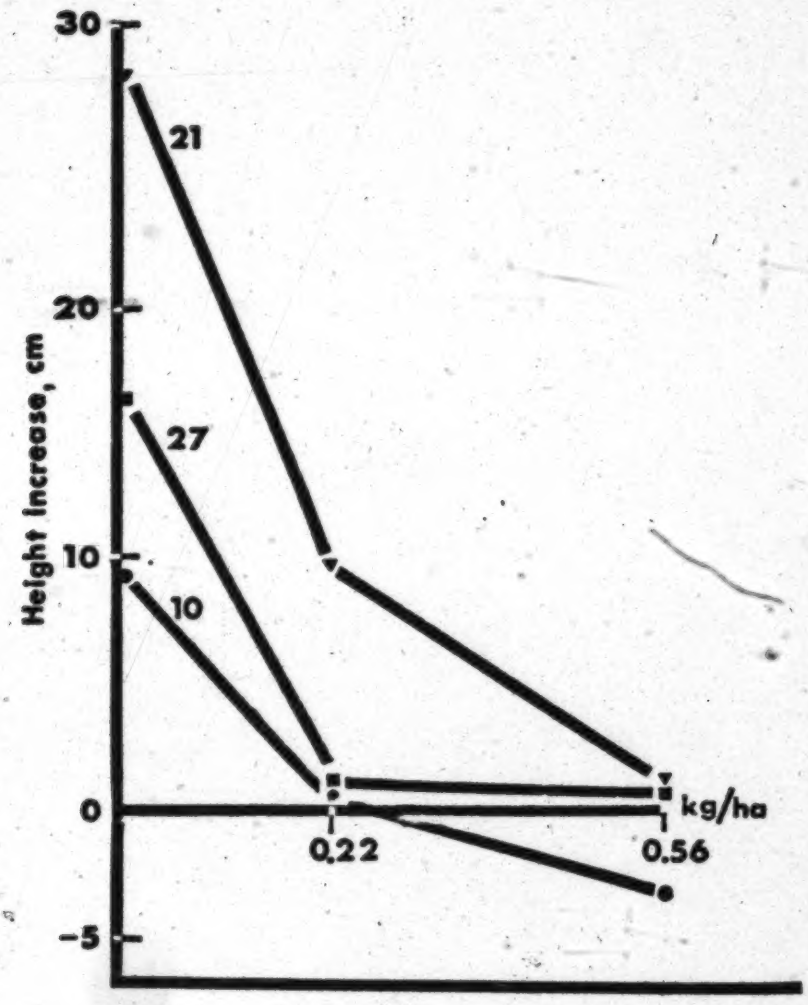


Figure 8. Growth (in cm) of Canada thistle shoots at 10, 21 and 27°C, four weeks after treatment with glyphosate at 0, 0.22 and 0.56 kg/ha.

Table 1. Number of new shoots set by Canada thistle at 10, 21 and 27°C after treatment with glyphosate at 0, 0.22, and 0.56 kg/ha.

Temperature	No. of new shoots per plant Glyphosate dosage, kg/ha 0	0.22	0.56
10°C	3.3 a*b	2.5 a	2.0 a
21°C	7.5 b	7.0 b	5.0 ab
27°C	13.3 c	16.0 c	15.3 c

* Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

Due to the increasing number of shoots produced at increasing temperature (Table 1) the dry weight of the entire plant increased (Table 2). Since glyphosate reduced top growth (Fig. 8) without influencing the number of shoots (Table 1) a reduction in dry weight would be expected. However, at the 0.22 kg/ha rate significant dry weight reduction occurred only at 21 and 27°C. The 0.56 kg/ha rate produced significantly greater reductions in dry weight than did the lower rate, at all three temperatures (Table 2). The reduction was slightly greater when the plants were growing well, at 21°C. Treatment of Canada thistle with 2,4-D, dicamba or picloram generally caused greater reduction in dry weight of the roots at higher temperatures (13, 41). The reduction was significant both

Table 2. Effect of temperature and glyphosate on the dry weight (d.w.) of Canada thistle plants sprayed at the bud stage and harvested four weeks later.

Temperature	Dry weight, g per plant Glyphosate dosage, kg/ha 0	0.22	0.56
10°C	20.5 a**	20.3 (0.1*) a	16.4 (20) b
21°C	21.8 a	18.3 (16) b	16.3 (25) c
27°C	24.3 a	21.9 (10) b	19.3 (21) c

* Numbers in parenthesis indicate percentage reduction in dry weight, based on corresponding control value.

** Means in the same row followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

from 16 to 21°C and from 21 to 27°C after treatment with 2,4-D, while for dicamba and picloram the reduction was only significant from 16 to 21°C.

8.1.5 Effects on secondary shoots along 45 cm long roots after glyphosate treatment of the end-shoot

In the greenhouse it was demonstrated that glyphosate does control secondary shoots via the root system. Shoots from the same root section could be partially to completely controlled by treating the end shoot (Table 3). Out of 20 plants tested in this experiment, eight which were considered representative were selected to demonstrate the results in Table 3. Glyphosate may cause injury to all secondary shoots (plant no. 1, 2, 5 and 6 in Table 3) or

some may lack injury symptoms (plant no. 3, 4, 7, and 8 in Table 3). Some secondary shoots evidently were bypassed by glyphosate. The variability occurred both when a taller and a smaller end-shoot were treated.

If glyphosate is translocated in the assimilate stream these results suggest that there may be no translocation of assimilates from the end-shoot to some or all secondary shoots. This hypothesis was tested by treating some of the glyphosate-treated end-shoots with $^{14}CO_2$. Forty eight hours later all shoots were harvested separately and the amount of ^{14}C-assimilates in each was determined (Table 4). The end-shoot at the time of $^{14}CO_2$ treatment, eight weeks after the glyphosate treatment, was suffering from glyphosate injury, but was still alive. In general no ^{14}C-assimilates were found in shoots without glyphosate symptoms (Table 4). Hence, the lack of glyphosate symptoms could be due to lack of assimilate translocation to these shoots, and the corresponding lack of glyphosate transport to them.

8.1.6 Root uptake

Glyphosate is a foliarly applied herbicide with little or no activity through the soil (2, 47, 68). The reasons for the lack of soil activity could be: (1) it is not taken up by roots, (2) it is inactivated in the soil by soil adsorption, microorganisms, or light, (3) downward leaching. An attempt will be made to elucidate some of these hypotheses.

Table 3. Canada thistle shoots on 45 cm root sections measured and scored for injury symptoms after foliar treatment (4 x 10 µl of 5000 ppm solution) of the end-shoot with glyphosate. 0 = no injury, 9 = complete kill.

		Height at treatment time, cm.				Injury score after three weeks					
	Plant No.	Treated end-shoot	Sec. shoot no.			Treated end-shoot	Sec. shoot no.*				
			1	2	3		1	2	3	4	5
Treated	1	7	0.5			4	9				
shoot	2	10	9	10		6	5	5			
tallest	3	8	8			4	0				
	4	14	12			5	0	6	7		
Treated	5	1	18	1	1	2	9	6	5	5	5
shoot	6	1	18			4	6	4			
smallest	7	5	12	24		4	0	0	0		
	8	7	27			6	0	5	5		

* For some plants more shoots emerged since treatment time.

Table 4. Translocation of ^{14}C-assimilates in Canada thistle shoots on 45 cm root sections, following treatment of the glyphosate treated end-shoot with $^{14}CO_2$. Same plants as in Table 3.

		^{14}C in root and secondary shoots, % of total*						
			Secondary shoot no.					
	Plant No.	Treated end shoot	1	2	3	4	5	Root	Total dpm recovered
Treated shoot taller	3	72 (4)	0 (0)	4	7	0		17	709,400
	4	58 (5)	0 (0)	2 (6)	4 (7)	25		11	698,300
Treated shoot smaller	7	94 (4)	2 (0)	0 (0)	0 (0)	1		3	347,000
	8	84 (6)	0 (0)	1 (5)	2 (5)	0.3	2	10.7	209,500

* Plants harvested 48 hours after $^{14}CO_2$ treatment. Glyphosate injury scores after three weeks are given in parenthesis.

Canada thistle grown in nutrient solution containing 1 ppm glyphosate showed herbicidal symptoms during the second week after treatment (Table 5). During the third week plants began to recover at 1 ppm. After the fourth week there was no sign of recovery at 10 ppm. The plants were killed within ten days at 100 ppm. Injury symptoms were identical to symptoms following foliar application (Fig. 4, page 32). Based on these results glyphosate is probably taken up by the roots, after which it inhibits plant growth. Hence the lack of herbicidal activity via the soil is not due to lack of root uptake. Glyphosate, therefore, must not be available to the roots in the soil.

Field results (10, 69) have indicated that the most lasting control of Canada thistle with glyphosate is obtained after application at a mature plant stage. Root uptake of glyphosate by Canada thistle in the greenhouse does not, however, show any age differences in sensitivity to the herbicide (Table 6). Increased injury was observed from the first to the third week. Plants treated at the earlier stage, 32 days old, showed symptoms later than plants treated at later stages, but after three weeks they showed more injury than the latter. The reduced control of younger plants under field conditions is not likely to be due to true tolerance, but rather to differences in translocation rate of glyphosate to the roots.

Table 5. Effects of glyphosate on 25-day old Canada thistle plants transferred to nutrient solution containing herbicide. Scores of foliar symptoms. 0 = no injury, 9 = complete kill.

Days after treatment	Injury scores Glyphosate, ppm 0	1	10	100
8	0		4.5 b	7.8 d
10	0	2.7 a*	6.4 c	9.0 e
25	0	1.7 a	6.7 cd	9.0 e

* Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

8.2 Greenhouse experiments with ^{14}C-labelled glyphosate

8.2.1 Recovery of ^{14}C from ^{14}C-glyphosate treated plants

Plants were treated with ^{14}C-glyphosate exactly as in field experiments to determine the amounts of ^{14}C-glyphosate taken up by the treated leaves and translocated to untreated plant parts. The recovery of ^{14}C was reduced from 91 percent of the dose after one day to 73 percent after one week (Table 7). After one day very little of the radioactivity had been absorbed by or translocated out of the treated leaves. After the first week 7.4 percent was recovered from untreated plant parts and 9.8 percent from the treated leaves. The remaining herbicide was still on the surface of the leaves and could be washed off.

Table 6. Effects of glyphosate on Canada thistle of increasing age, transferred to nutrient solution containing the herbicide. Scores of foliar symptoms. 0 = no injury, 9 = complete kill.

Age at treatment time, days	Days after treatment: 7		14		21	
	Glyphosate, ppm: 1	10	1	10	1	10
32	0 a*	1.3 b	1.7 a	5.0 c	3.7 a	8.0 c
46	0.7 ab	3.3 c	1.7 a	5.0 c	3.3 a	7.3 cd
74	0 a	4.0 c	0.7 b	5.0 c	1.7 b	6.0 e
88	1.0 b	3.7 c	0.7 b	5.3 c	2.0 b	6.3 de
Average	0.4	3.1	1.2	5.1	2.7	6.9

* Means for the same scoring day followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

Table 7. Recovery of ^{14}C from Canada thistle plants, 20 cm tall, up to one week after treatment with ^{14}C-glyphosate.

Sampling Time	^{14}C, % of total*				
	Rinse of treated leaves	Treated leaves	Rest of plant	Total dpm recovered	% Recovery
0 hr.	99.9 a**	0.1 a	0 a	243,000	100 a
6 hr.	89.6 b	2.6 b	0.4 b	225,000	93 b
24 hr.	88.6 b	2.2 b	0.5 b	222,000	91 b
1 week	55.6 c	9.8 c	7.4 c	177,000	73 c

* Figures are means of three replicates.

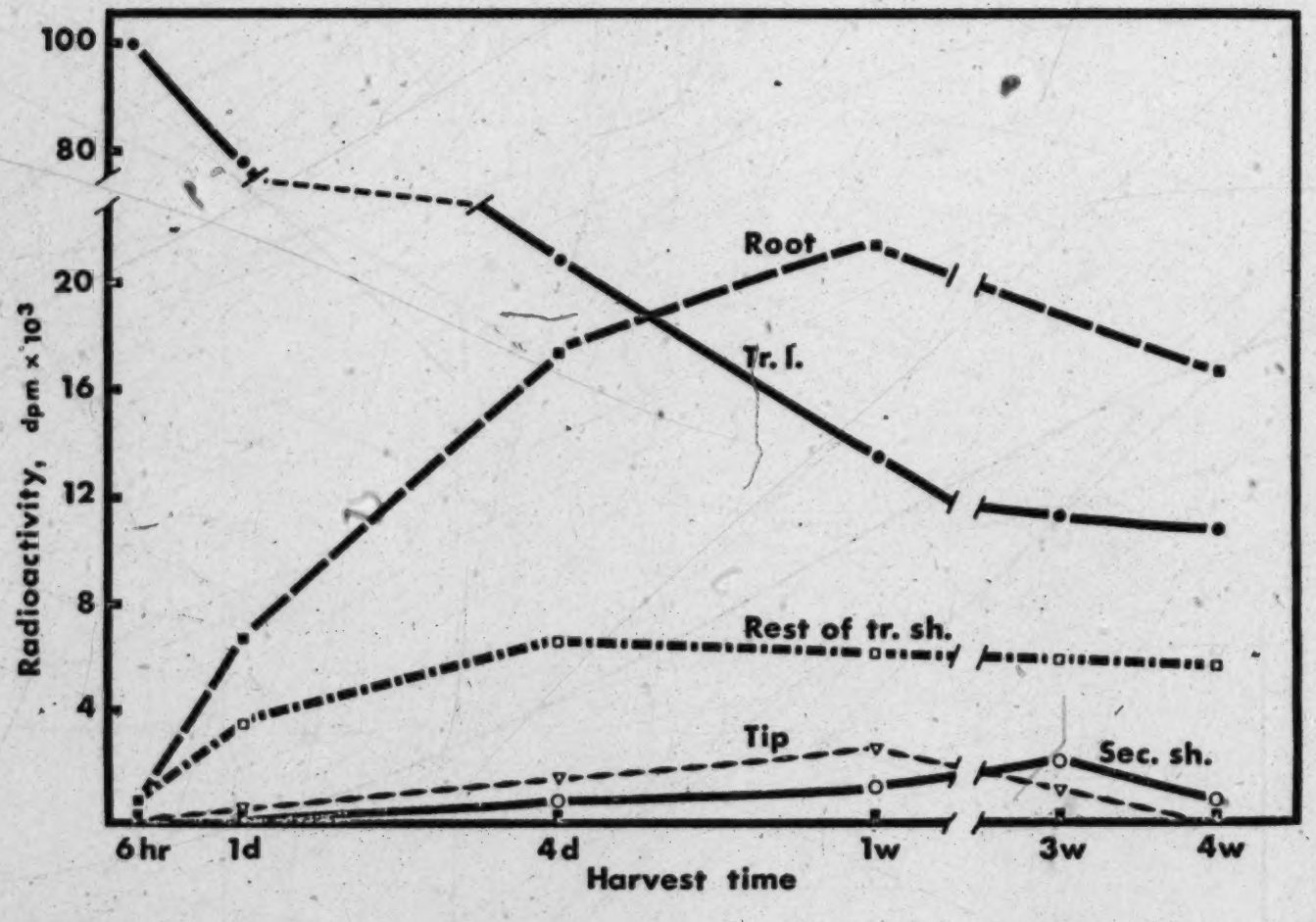
** Means in the same column followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

8.2.2 Translocation velocity of ^{14}C to untreated plant parts after ^{14}C-glyphosate treatment

The recovery of total ^{14}C decreased rapidly during the first four days following application and more slowly for the next three or four weeks. The decrease in recovered radioactivity from the treated leaves (Fig. 9) is possibly due to a loss of activity from the surface of the treated leaves (Table 7) to the environment. The lower recovery of ^{14}C in this experiment (Fig. 9) than in previous results (Table 7) was considered due to wash-off of the ^{14}C-glyphosate in this experiment.

Glyphosate symptoms appeared on young leaves and growing tips, which may lead one to conclude that glyphosate was translocated to these sites along with the

Figure 9. Distribution and recovery of ^{14}C in Canada thistle at different times after application of ^{14}C-glyphosate to four mature leaves of one shoot.



% Recovery	50	45	24	22	15	17

assimilate stream. Auxin-type herbicides follow this pattern (65). Data in Fig. 9 show, however, that not much radioactivity had accumulated in the tip of the treated shoot. This was also borne out in autoradiographic studies, which will be described later (8.4.3). The rest of the treated shoot contained significantly higher amounts of radioactivity than the secondary shoots. However, on a per gram dry weight basis the rest of the treated shoot may contain no more ^{14}C than the secondary shoots. Radioactivity in the roots accumulated rapidly during the first four to seven days after treatment, and then decreased slightly. The percentage of total activity in the rest of the treated shoot, the tip, and the roots increased rapidly during the first four days and to a lesser extent up to seven days after treatment. The percentage recovered from each plant part then stayed constant until four weeks, except for the tip of the treated shoot where a decline began after one week.

Leaves of six untreated plants, grown among the treated plants in the growth chamber, contained detectable amounts of radioactivity after four weeks (460, 580, 1480, 430, 0 and 161 dpm per plant). No activity could be detected one week after treatment and no radioactivity was found in roots. It seems likely that these plants had taken up the radioactivity as $^{14}CO_2$ from the atmosphere in the growth chamber. The $^{14}CO_2$ must then have been given off by the treated plants. Some metabolism of ^{14}C-glyphosate

was, therefore, likely to have taken place in Canada thistle.

8.2.3 Root exudation

Two plants were grown together in pots or in Erlenmeyer flasks containing nutrient solution. One plant in each pot or flask was treated with six 10 µl drops of a 5000 ppm glyphosate solution. The treated plants died and were removed from the nutrient solution during the third week after treatment. The untreated plants showed no herbicidal injury symptoms four weeks after treatment. Possible root exudation was also studied by foliarly treating five-week old plants grown in nutrient solution. They were treated with 0.1 and 0.2 µCi of ^{14}C-glyphosate and 1 ml aliquots of the nutrient solution were sampled after 0, 6, 12, 24, 36 and 48 hours, 3 and 4 days, 1 and 2 weeks. No ^{14}C was detected in the nutrient solution at any of the sampling times.

These results indicate that there was no root exudation of intact glyphosate or of ^{14}C-metabolites, and no exchange of toxic amounts of metabolites via the root system of individual plants. Root exudation of glyphosate metabolites containing no ^{14}C is possible, however. If so, these metabolites could be:

1. non toxic
2. toxic, but in too low a concentration to be harmful
3. toxic, but degraded in the nutrient

solution before reabsorption could take place

4. unavailable to plant roots.

8.3 Field experiments with unlabelled glyphosate

8.3.1 Post-harvest application

A barley field, heavily infested with Canada thistle, was harvested August 16. Plots 2.44 m by 4.57 m, with four replicates, were then staked out and sprayed with 0, 2.2 and 4.5 kg/ha of glyphosate after 0, 33 and 55 days. The last treatment was applied after 11 consecutive nights of frost. The temperature at the time of the two latest applications was 7 to 8°C. Application of glyphosate immediately after harvest did not significantly reduce the number of shoots emerging the following spring (Table 8). This was expected, because there were very few leaves left on the shoot stumps or young shoots after harvest. There was some Canada thistle regrowth at the time the later treatments were applied, and these applications significantly reduced the number of emerging shoots the following spring (Table 8). Application in September and October resulted in the same degree of control of Canada thistle. Increase of the rate of glyphosate from 2.2 to 4.5 kg/ha did not increase control. These results suggest that late fall application of glyphosate on regrowth of Canada thistle can reduce the number of shoots emerging the following spring by 60 to 70 percent.

An infestation of dandelion (Taraxacum officinale)

Table 8. Effect of post-harvest application of glyphosate on Canada thistle.

Treatment time, days after harvest	Treatment date	Dose kg/ha	Shoots per m^2*
0	Aug. 16	0	20 a**
		2.2	15 a
		4.5	13 ab
33	Sept. 18	2.2	7 bc
		4.5	6 c
55	Oct. 10	2.2	6 c
		4.5	6 c

* Shoots counted June 12th the following spring.

** Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

in these plots was also reduced the following spring due to the glyphosate treatments.

8.4 Field experiments with ^{14}C-glyphosate

In field experiments Canada thistle was treated at four growth stages, namely: 14 cm, 22 cm, bud, and C-4 cm (4.2, page 19). Mature plants (22 cm and bud) in this experiment were not as tall as would be expected under normal field conditions. This was due to lack of competition and cold weather conditions during the month of July. Extensive growth of side and secondary shoots took place; plants at the C-4 stage produced up to 45 secondary shoots. The secondary shoots produced were separated into shoot

Table 9. Recovery of ^{14}C from ^{14}C-glyphosate treated Canada thistle plants in the field.

Sampling time	^{14}C, % of total		Total dpm recovered	% Recovery
	Treated leaves	Rest of plant		
1 hour	55.9 a*	0.6 a	112,900	56.5 a
1 day	30.4 b	7.3 b	75,400	37.7 ab
1 week	8.5 c	10.5 b	38,000	19.0 bc
4 weeks	2.0 c	5.5 b	14,900	7.5 c

* Means in the same column followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

categories at the time of harvest. Category I was the treated shoot. Categories II and III were the bigger secondary shoots which had emerged from the planted root section. Categories IV to VI contained the rest of the secondary shoots divided equally among them. All shoots in a shoot category were analysed together.

Many plants became infested with Painted Lady butterfly larvae (Pyrameis cardui L.) (62). All larvae visible were removed by hand.

8.4.1 Recovery of ^{14}C from treated and untreated plant parts

The amount of ^{14}C recovered from field-grown plants decreased rapidly with time after treatment (Table 9) and was lower than the amount recovered from plants grown in the greenhouse (Table 7, page 47). At most 10.5 percent

(i.e. approximately 0.01 µCi or 20,000 dpm) of the 0.1 µCi applied was recovered from untreated plant parts. Four weeks after treatment the amount of ^{14}C recovered from untreated plant parts was reduced to 10,900 dpm or 5,5 percent of the dose applied. This limited amount of activity was detected with difficulty by autoradiography.

8.4.2 Bypassing of secondary shoots

From greenhouse experiments it was evident that when an end-shoot was treated with glyphosate, some of the secondary shoots along a root section could be bypassed in the translocation of glyphosate. In field studies, some secondary shoots of some plants lacked radioactivity after ^{14}C-glyphosate treatment, while ^{14}C was present in all secondary shoots of other plants (Table 10). This phenomenon occurred irrespective of treatment stage. The data in Table 10 are from plants harvested one week after treatment, since they contained the most radioactivity (Table 9, page 54). When no radioactivity was found in shoots in categories II or III while it was found in the other shoot categories, the former were considered bypassed.

8.4.3 Distribution of ^{14}C in ^{14}C-glyphosate treated Canada thistle plants

The distribution of radioactivity is shown in Fig. 10. Results are similar to those obtained in the greenhouse studies (Fig. 9, page 48). However, in the field more activity was recovered from the secondary shoots and less from the roots. This could have resulted from the increased

Table 10. Percentage of total ^{14}C recovered from different shoot categories and roots one week after foliar treatment of Canada thistle plants with ^{14}C-glyphosate in the field. Selected examples.

	Plant No.	Shoot categories: Treated leaves	Rest of tr.shoot	II	III	IV	V	VI	Roots
Bypassing	1	79	12	4	0	0	0	4	1
	2	57	31	6	0	0	6		0
	3	69	23	0	0	0	1	2	5
	4	74	21	1	0	0	1	0	3
No Bypassing	5	76	13	3	2	1	1	3	1
	6	37	11	5	7	2	22	5	11
	7	56	25	4	3	4	2	3	3
	8	26	36	15	5	7	3	6	8

number of secondary shoots set by the field plants. The ratio of secondary shoots in the field to those in the greenhouse was 5:1.

The amount of ^{14}C in the treated leaves decreased throughout the four-week period. In the first 24 hours after treatment there was a rapid increase in the amount of radioactivity detected in the rest of the treated shoot, the secondary shoots, and the roots. This increase continued at a reduced rate until one week after treatment, after which there was a gradual decline. The decrease in activity in the plant indicates a loss of ^{14}C. Two possible explanations exist. Firstly, the ^{14}C could be incorporated into plant constituents which are insoluble in the extraction medium. This hypothesis was tested by counting samples of the extracted tissue in a liquid scintillation counter. No increase in incorporation was detected over the four week period. Secondly, one or more metabolites containing ^{14}C could be lost to the surrounding environment. One such metabolite is likely to be $^{14}CO_2$ (8.2.2, page 47).

Plants from all four treatment stages in this field experiment were autoradiographed. As shown in Table 9 (page 54) the amount of radioactivity translocated in the plants was at most 10 percent of the dose or 20,000 dpm. This amount of activity distributed in a plant was only detected with difficulty by autoradiography. Figure 11 consists of two of the best autoradiographs of two plants harvested one day following treatment. The spots on the treated leaves are the areas where the herbicide was applied.

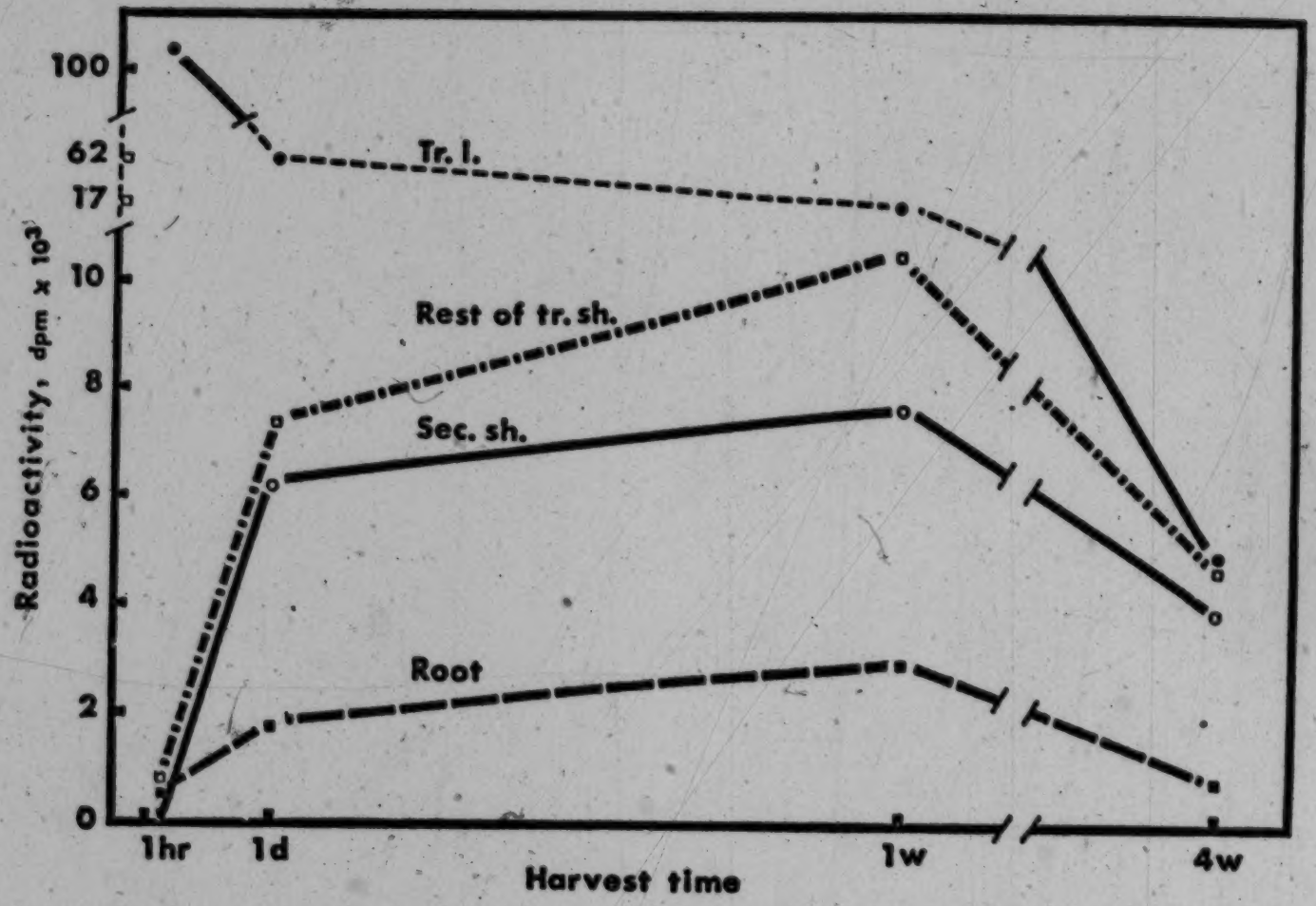


Figure 10. Distribution of ^{14}C in field grown Canada thistle plants at different times after application of 14C-glyphosate. Average of plants at all treatment stages.

Very little activity was present in the tip of the treated shoot, but the older leaves had some labelling. Secondary shoots were labelled uniformly in all leaves (Fig. 11). A source-to-sink movement of glyphosate does not adequately explain the observations, since there was no accumulation of activity in the active sinks of growing points.

8.4.4 Translocation of ^{14}C at four growth stages

The data for the secondary shoots were pooled for plants at each treatment stage, since they all showed the same trend and were not significantly different. Some of the secondary shoots lacked radioactivity as previously mentioned (8.4.2). The data from plants at the different treatment stages showed great variability and as a result any significant trends may be hidden (Table 11). In the treated leaves the amount of radioactivity recovered declined rapidly with time. No explanation can be offered for the low recovery from the treated leaves of plants at the C-4 stage after one hour. For the plants treated at the 14 cm stage, the amount of ^{14}C in the rest of the treated shoots remained constant over the four week period, while in the secondary shoots and roots it increased. For plants treated at the 22 cm stage the amount of radioactivity in the untreated plant parts increased up to one week and subsequently decreased. Plants at the bud stage had the greatest amount of ^{14}C present in the untreated plant parts after one day, followed by a decrease up to four weeks after treatment. In plants at the C-4 stage the amount of radio-

Figure 11. Distribution of ^{14}C in Canada thistle one day after ^{14}C-glyphosate application. Treated leaves are arrowed. Left: plant mounts, Right: autoradiograms.

Table 11. Distribution of radioactivity, dpm, in four plant parts at four treatment stages and four harvest times.

Plant Part	Treatment Stage*	Harvest Time			
		1 Hour	1 Day	1 Week	4 Weeks
Treated leaf	14	113,100 ab**	40,100 a	13,500 a	4,000 a
	22	117,500 ab	59,600 a	23,900 a	2,600 a
	bud	149,900 a	77,600 a	20,100 a	5,500 a
	C-4	66,800 b	64,600 a	10,400 a	***
Rest of treated shoot	14	900 a	7,300 ab	6,600 a	7,500 a
	22	1,700 a	12,800 b	12,800 ab	2,500 a
	bud	0 a	7,900 ab	8,000 ab	3,500 a
	C-4	200 a	1,700 a	13,700 b	
Sec. shoot	14	0 a	1,200 a	3,700 a	10,700 a
	22	0 a	7,400 a	17,400 b	2,000 b
	bud	0 a	7,500 a	3,500 a	2,000 b
	C-4	0 a	6,900 a	6,500 ab	
Root	14	0 a	1,400 a	800 a	4,100 a
	22	1,000 a	1,400 a	7,300 a	500 b
	bud	0 a	2,400 a	1,300 a	0 b
	C-4	700 a	1,200 a	2,400 a	

* Plants were treated when 14 cm tall (14), 22 cm tall (22), at the bud stage (bud), and regrown to 4 cm after being cut down to ground level at the early bud stage (C-4).

** Means in the same column for the same plant part followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

*** For plants at the C-4 treatment stage samples could not be harvested four weeks after treatment due to an early killing frost.

activity in untreated plant parts increased during the first week. Data for the four week harvest were not obtained.

Hodgson (38) describes the early bud stage as having the greatest downward flow of assimilates. If glyphosate, like some other herbicides (1, 9, 23, 48, 49, 65) moves with the assimilate stream, the results in Table 11 would be in agreement with Hodgson's results (38). At the bud stage, plants translocated the ^{14}C most rapidly, followed by plants at the 22 cm stage. However the radioactivity also disappeared most quickly from plants at these two stages, especially in the roots. The decrease in radioactivity in all plant parts after four weeks suggests that the ^{14}C is lost from the plants, either as intact glyphosate or as a metabolite. One possible metabolite is $^{14}CO_2$. Some of the $^{14}CO_2$ is likely to be reassimilated in the leaves. This may account for the higher levels of ^{14}C in the shoots as compared to the roots after four weeks. Also, the ^{14}C present in the roots may be translocated to the shoots or lost to the soil. Loss to the soil, however, is considered negligible since no ^{14}C was found in nutrient solution, in which ^{14}C-glyphosate treated Canada thistle plants were grown (8.2.3). Possible loss of $^{14}CO_2$ from the roots could not be detected in this experiment.

9. Leafy spurge

9.1 Effects of treatments with unlabelled glyphosate in greenhouse experiments

9.1.1 Symptoms on shoots and leaves

Glyphosate symptoms observed on leafy spurge were similar to those observed on Canada thistle. The first symptoms occurred as chlorosis of the young emerging leaves (Fig. 12), although the veins remained green. Leaves emerging after treatment had difficulty unfolding. Following spray application the younger leaves showed symptoms one to two days earlier than the older leaves. Root application of glyphosate caused the younger leaves to show symptoms four to five days before the oldest, while the intermediate leaves stayed green.

9.1.2 Control of leafy spurge

a. Spray treatments

The rate of glyphosate required for leafy spurge control in the greenhouse (2.2 kg/ha) (Table 12) was at least four times that required for Canada thistle (0.56 kg/ha) (Fig. 2, page 28). Six-week old plants were killed six weeks after spraying with 2.2 kg/ha. Six-month old plants were not killed by this treatment, but ceased growth after six weeks. Young shoots which emerged after spraying had necrotic tips. The higher rate (4.5 kg/ha) did not control leafy spurge any quicker than the 2.2 kg/ha rate.

b. Dipping

All six-week old plants had only one shoot (Table 13).

A B C

Figure 12. Effects of sublethal doses of glyphosate on leafy spurge. A. 2 kg/ha, foliar applied, B. control, C. 10 ppm root applied. Plants were treated when five weeks old and photographed 10 days later.

Table 12. Control of leafy spurge with glyphosate.

Scores: 0 = no injury, 9 = complete kill.

Scoring time	Treatment stage: 6 weeks old, Dose kg/ha 0	2.2	4.5	6 months old, Dose kg/ha 0	2.2	4.5
3 weeks	0	6 a*	7 a	0	4 a	4 a
6 weeks	0	9 b	9 b	0	6 b	6 b

* Means for plants at the same treatment stage followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

The amount of glyphosate solution retained by the leaves was low due to poor wettability. The solution retained by the leaves ran to the tip and formed a droplet which slowly evaporated. This resulted in leaf necrosis. To kill six-week old plants a 5000 ppm solution was required (Table 13). None of the well established older plants was killed and symptoms did not change significantly from the second to the sixth week following any of the treatments (Table 13). After dipping of the main shoot in a 1000 ppm solution of glyphosate secondary shoots showed injury symptoms after two weeks. However, after six weeks the secondary shoots had recovered .

Results of both the spraying and dipping treatment of leafy spurge suggest that poor spray retention may partially account for the poor control in the greenhouse.

Table 13. Control of leafy spurge following dipping of the tallest shoot in glyphosate solution. Scores: 0 = no injury, 9 = complete kill.

Treatment time	Scoring time	Shoot	Glyphosate concentration, ppm 0	1,000	5,000	10,000	20,000
6 weeks old	2 weeks	treated	0	3 a*	4 a	6 b	6 b
	6 weeks	treated	0	4 a	9 b	9 b	9 b
6 month old	2 weeks	treated	0	4 a	5 a	7 b	7 b
		secondary	0	3 a	3 a	6 b	6 b
	6 weeks	treated	0	3 a	5 b	7 c	7 c
		secondary	0	0	3 a	4 a	7 b

* Means in the same row followed by the same letters are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

Additional surfactant was added to the glyphosate solution, which already contained some surfactant, in an attempt to improve the spray retention and thereby the control.

c. Addition of surfactants to the spray solution

Three to four weeks after spraying glyphosate, there were no differences in the degree of injury between the 1.1 kg/ha and 2.2 kg/ha rates (Table 14 and Table 12, page 66). Addition of either Atplus 411F or Tween 20 to the glyphosate spray solution did not improve leafy spurge

Table 14. Leafy spurge control with glyphosate containing additional surfactant

Glyphosate kg/ha	% surfactant	Scoring* time			Increase in height and shoot number after six weeks**	
		2 wks.	4 wks.	6 wks.	Height cm	No. of shoots
Tween 20						
0	0	0 a***	0 a	0 a	18.3 a	2.0 a
0	0.5	0 a	0 a	0 a	15.3 a	4.0 ab
1.1	0	3.7 b	4.3 b	5.7 b	1.0 b	8.3 c
1.1	0.1	3.3 b	1.7 c	1.3 c	15.7 a	5.0 bc
1.1	0.5	1.7 c	5.0 b	5.3 b	4.3 b	6.0 bc
Atplus 411F						
0	0	0 a	0 a	0 a	15.2 a	1.0 a
0	0.5	0 a	0 a	0 a	13.3 a	1.3 a
1.1	0	3.7 b	4.7 b	5.3 b	3.3 b	2.6 a
1.1	0.1	3.7 b	4.7 b	5.7 b	4.0 b	2.3 a
1.1	0.5	4.0 b	5.0 b	5.3 b	4.3 b	3.0 a

* 0 = no injury, 9 = complete kill

** Plants were three months old when treated

*** Means in the same column for the same surfactant followed by the same letter were not significantly different at the 5% level using Duncan's Multiple Range Test (21).

control. Visible injury symptoms increased during the first six weeks following spraying, but no plants were killed, and

after two months recovery was evident (Table 14). Addition of 0.1 percent Tween 20 partially removed the herbicidal effect. Plants given this treatment increased in height as much as the control plants. Addition of 0.5 percent Tween 20 restored the controlling effect which did not differ significantly from the effect of the commercial glyphosate alone. No explanation can be offered for the partial removal of the herbicidal effect by Tween 20. Information on the surfactant already present in the commercial glyphosate formulation (47) was not available.

Leafy spurge sprayed with glyphosate has a tendency to set more shoots than control plants (Table 14). This effect was clearly demonstrated on Canada thistle (Fig. 2, page 28).

9.1.3 Effects on secondary shoots along 30 cm long roots from glyphosate treatment of the end-shoot

Shoots along a root section of leafy spurge exhibited herbicidal symptoms following glyphosate treatment of a terminal shoot (Table 15). The plants in this experiment had fewer secondary shoots than the Canada thistle (8.1.5) and no bypassing of these was noted (Table 15). Leafy spurge treated with the same dose as Canada thistle displayed less injury although symptoms did occur on all shoots.

9.1.4 Root uptake

Leafy spurge grown in nutrient solution containing

Table 15. Leafy spurge evaluated for injury symptoms on each shoot after foliar treatment of one end-shoot with glyphosate (4 x 10 µl of a 5000 ppm solution)

Height, cm			Injury score after 2 weeks		
Treated shoot	Sec.sh.no.		Treated shoot	Sec.sh.no.	
	1	2		1	2
29	23		3	3	
15	15	20	2	1	1
12	25	25	3	1	2
20	33		3	2	
14	23		3	1	
14	25	19	5	1	1
20	28		4	3	
Average			3.3	1.9	1.3

10 ppm glyphosate showed herbicidal symptoms after three to four days, and plants grown at 1 ppm began to show symptoms on the fourth day (Table 16). After four weeks, plants began to recover at 1 ppm, while there was no sign of recovery at 10 ppm. Following root uptake leafy spurge showed glyphosate symptoms quicker than Canada thistle, and the degree of control was comparable for the two species. The lack of control of leafy spurge after spray application is therefore likely to be due to lack of uptake of glyphosate.

Lack of uptake could be caused by poor spray retention as mentioned earlier (9.1.2).

Table 16. Effects of glyphosate on leafy spurge grown in nutrient solution containing the herbicide. Scores of foliar symptoms: 0 = no injury, 9 = complete kill.

Scoring time*	Glyphosate concentration, ppm		
	0	1	10
4 days	0	0.7 a**	3.0 bc
11 days	0	2.0 b	4.0 cd
24 days	0	4.3 d	6.7 e
30 days	0	3.0 bc	7.7 e

* Plants were six weeks old when treated.

** Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

9.2 Recovery of ^{14}C from ^{14}C-glyphosate treated plants

Uptake and translocation of glyphosate by the treated leaves was studied. Initially, it was intended to carry out this experiment with and without addition of surfactant. Poor wettability of leafy spurge leaves, however, made application of 5 µl droplets of ^{14}C-glyphosate solution without surfactant impossible.

At high relative humidity (RH) significantly less radioactivity could be washed off the treated leaves after

twelve or more hours, than at low RH (Table 17). Considerably more uptake by the treated leaves at high RH accounts for the smaller amount of ^{14}C-glyphosate present on these leaves. At low RH, uptake of ^{14}C-glyphosate increased during the first week after application. Some ^{14}C-glyphosate was translocated to the rest of the shoots and the root within 12 hours after application, with accumulation occurring from the second to the seventh day. The accumulation of radioactivity indicates continued translocation out of the treated leaves during the first week. At high RH the maximum amount of ^{14}C-glyphosate was in the treated leaves after 12 hours while it declined during the following week. This decline is likely to be due to translocation of ^{14}C-glyphosate out of the treated leaves to the rest of the shoots in which the amount of radioactivity increased during the first 48 hours. Translocation to the roots took place within 12 hours and to a greater extent at high than at low RH. The ^{14}C-glyphosate and possibly ^{14}C-metabolites accumulated in the roots for the first week. Improved uptake and translocation of glyphosate at high RH would be expected to give better control as has been found by Wills (72).

The greater translocation of ^{14}C-glyphosate to the shoots and roots at high RH is considered due to greater and more rapid uptake of glyphosate at high RH. Similar effects have been found with other herbicides (6, 12, 53, 65).

Table 17. Recovery of ^{14}C from leafy spurge treated with ^{14}C-glyphosate containing surfactant.

Harvest time	% of total ^{14}C-glyphosate: Rinse of tr.l.	Treated leaf	Shoots	Roots	Total dpm x 10^3 recovered	% Recovery
Low RH						
0 hr.	99.8 a*	0.2 a	0 a	0 a	629	100 a
12 hr.	91.5 ab	4.6 ab	3.5 ab	0.5 a	480	76 b
24 hr.	92.8 ab	3.6 ab	3.0 ab	0.7 a	464	74 b
48 hr.	86.2 bc	9.2 b	2.7 ab	1.9 ab	502	80 b
1 week	76.1 c	8.3 b	10.0 b	8.3 b	495	79 b
High RH						
12 hr.	28.7 a	59.4 a	11.8 a	1.0 a	472	75 a
24 hr.	13.4 a	53.1 a	30.1 ab	3.4 ab	470	75 a
48 hr.	12.3 a	34.7 b	41.4 b	11.5 b	430	68 a
1 week	11.3 a	20.8 b	37.6 b	30.4 c	482	77 a

* Means in the same column for the same RH followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test (21).

10. Soil adsorption of ^{14}C-glyphosate

Earlier experiments (8.1.6) demonstrated that glyphosate, though inactive through the soil, can be taken up by Canada thistle and leafy spurge roots from nutrient solution. Soil adsorption and degradation are the most likely causes of inactivation (46, 61, 66). The adsorption of ^{14}C-glyphosate was studied by adding 0, 1, 2, 4, 8 and 16 percent (W/V) of an unsterilized 3:2:1 soil mixture (Malmo clay loam:peat:sand) to 50 ml of distilled water containing 0.1 µCi of ^{14}C-glyphosate. The mixture was shaken for 20 minutes and filtered. One ml aliquots of the filtrate then were taken for liquid scintillation counting. The results shown in Fig. 13 indicate rapid initial soil adsorption of glyphosate by the one to four percent soil suspension. The adsorption is only slightly enhanced by doubling the amount of soil from 8 to 16 percent. At the latter point only about ten percent of the glyphosate is in solution. The results are in agreement with the strong soil adsorption reported by others (46, 61, 66). McPhee (46) found that glyphosate at 100 ppm mixed in PDA agar inhibited the growth of Fusarium fungi. Addition of sterilized soil to the glyphosate containing agar (100 ppm) removed the retardation of fungal growth. Sprankle et al. (66) found a correlation between the levels of phosphate in soil and its capacity for glyphosate adsorption.

Microbiological breakdown of glyphosate is also

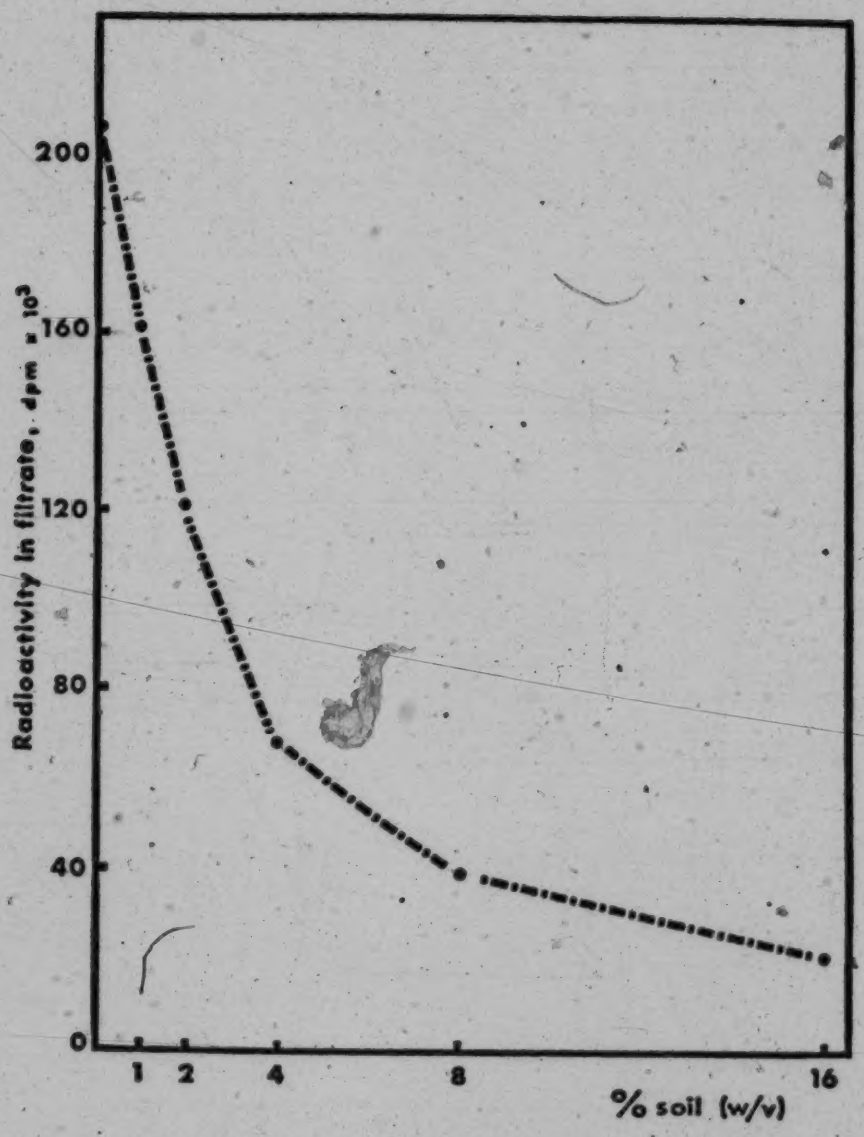


Figure 13. Soil adsorption of ^{14}C-glyphosate. Fifty ml of water containing 0.1 µCi of ^{14}C-glyphosate was shaken with increasing amounts of soil for 20 min. and filtered.

a source of inactivation (66). However, microbiological breakdown during the 20 minute experimental time is considered minimal. Sprankle et al. (66) found 10 to 49 percent breakdown in 28 days. They also found that the rate of degradation of glyphosate in the soil was independent of the capacity of the soil to adsorb glyphosate.

11. Translocation of $^{14}CO_2$ assimilates (field studies)

Results from previous experiments indicated that when a terminal shoot of Canada thistle was treated with glyphosate, some of the secondary shoots were bypassed. If one assumes that glyphosate, like many other herbicides (1, 48, 49, 65, 68), is phloem-translocated along with the assimilate stream, a knowledge of assimilate translocation patterns could provide useful information in predicting translocation patterns of glyphosate. Therefore, the translocation of ^{14}C-assimilates in Canada thistle, leafy spurge and toadflax was investigated in the field.

No percentage recovery data of ^{14}C are provided because of variability in total $^{14}CO_2$ fixed by plants of different size and leaf area.

11.1 Canada thistle

Canada thistle plants containing many secondary shoots were treated with $^{14}CO_2$ at various growth stages (4.2, page 19). All shoots present at treatment time were numbered; #1 was the largest (treated) shoot, while the secondary shoots were numbered consecutively with distance

from #1. Shoots #2 to 4 were the largest secondary shoots. Only representative plant parts were autoradiographed.

Of the $^{14}CO_2$ assimilates 3,000,000 to 29,000,000 dpm per plant was recovered. No differences in the distribution of ^{14}C after 48 hours were found between plants treated at different growth stages (Table 18). The main portion of the ^{14}C (68 to 85 percent) remained in the treated shoot while 5 to 15 percent was recovered from the roots (Table 18, Fig. 14). Secondary shoots 2 to 4 sometimes imported label from the treated shoot and sometimes did not, thus indicating bypassing. However, other secondary shoots numbered 5 or higher usually contained some label, indicating that they were not bypassed. Exceptions to this are plants (b) and (c) in Fig. 14 where shoots #7 and 9, respectively were bypassed. Plant (d) at the C-4 stage did not translocate any assimilates to other shoots. This could be because the treated shoots are barely self-supporting. The ^{14}C-assimilates translocated to the secondary shoots were concentrated mainly in the younger leaves (Fig. 14) as would be expected since these are sinks for assimilates. Similar translocation patterns were not obtained with ^{14}C-glyphosate. The herbicide often bypassed secondary shoots of all growth stages (Table 3, page 42 and Table 10, page 56). However, when translocated to secondary shoots it did not concentrate in the younger leaves, but was evenly distributed in the shoot (Fig. 11, page 60). This observation indicates that glyphosate may not follow the assimilate

Table 18. Distribution of ^{14}C-assimilates in Canada thistle 48 hours after treatment with $^{14}CO_2$.

Growth stage of treated shoot; cm	^{14}C recovered, % of total						
	Treated shoot	Shoot no.					
		2	3	4	5-8	9-	Root
14	76	0	5	3	3	4	9
14	78	0.1	0.9	1	1	9	10
22	81	0	2	2	3	5	7
22	77	8	0	0.5	2.5	7	5
bud (30)	82	2	1	2	3	4	6
bud (30)	74	6	0	0.3	4.7	6	9
C-4*	68	0	9	6	3		14
C-4	85	6	0.5	0.2	1	1	6.3

* Cut down to ground level at the bud stage and treated after regrowth to 4 cm.

Figure 14. A and B. Distribution of ^{14}C-assimilates in Canada thistle plants at four growth stages (14 cm, 22 cm, bud, C-4 cm), 48 hours after $^{14}CO_2$ application to a main shoot (tr.). Representative plant parts only. Left: plant mounts (shoot numbers are indicated on the photographs). Right: autoradiograms.

14
3
4
5
6
2
17
3
5
6
17
2
22
12
13
14
5
7
6
11
17
2
11-14
A

BUD
C-4
B

stream completely.

Shoots at nearly the same growth stage as the treated shoot (Table 18, Fig. 14) may also import some ^{14}C-assimilates. Such import by large secondary shoots would not be expected, since these should be self-supporting. According to Hodgson (38) and Müller (49) 5 cm tall shoots are self-supporting and 10 to 15 cm tall shoots export assimilates. Hence the import by the larger secondary shoots should not be caused by a lack of food materials for normal growth.

11.2 Leafy spurge

Leafy spurge did not establish many secondary shoots during the three month growing season. Of the $^{14}CO_2$ assimilated 600,000 to 8,000,000 dpm per plant was recovered. In all cases the treated shoot retained most of the assimilated radioactivity (Table 19). Treatment of plants at different stages did not influence the distribution of ^{14}C-assimilates. The translocated ^{14}C-assimilates accumulated in the roots, especially in shoot buds. Hunter et al. (40) obtained similar results with 2,4-D. Some larger secondary shoots did not import any radioactivity, as found with Canada thistle. In one case, however, as much as 22 percent was recovered from a large secondary shoot (Table 19, 14 cm). It is evident from Fig. 15 (plants a, b, e) that lack of radioactivity in secondary shoots can be due to bypassing. Similarly plants d and f indicate that there may be a complete absence of assimilate translocation to a root portion. Small shoots that did not

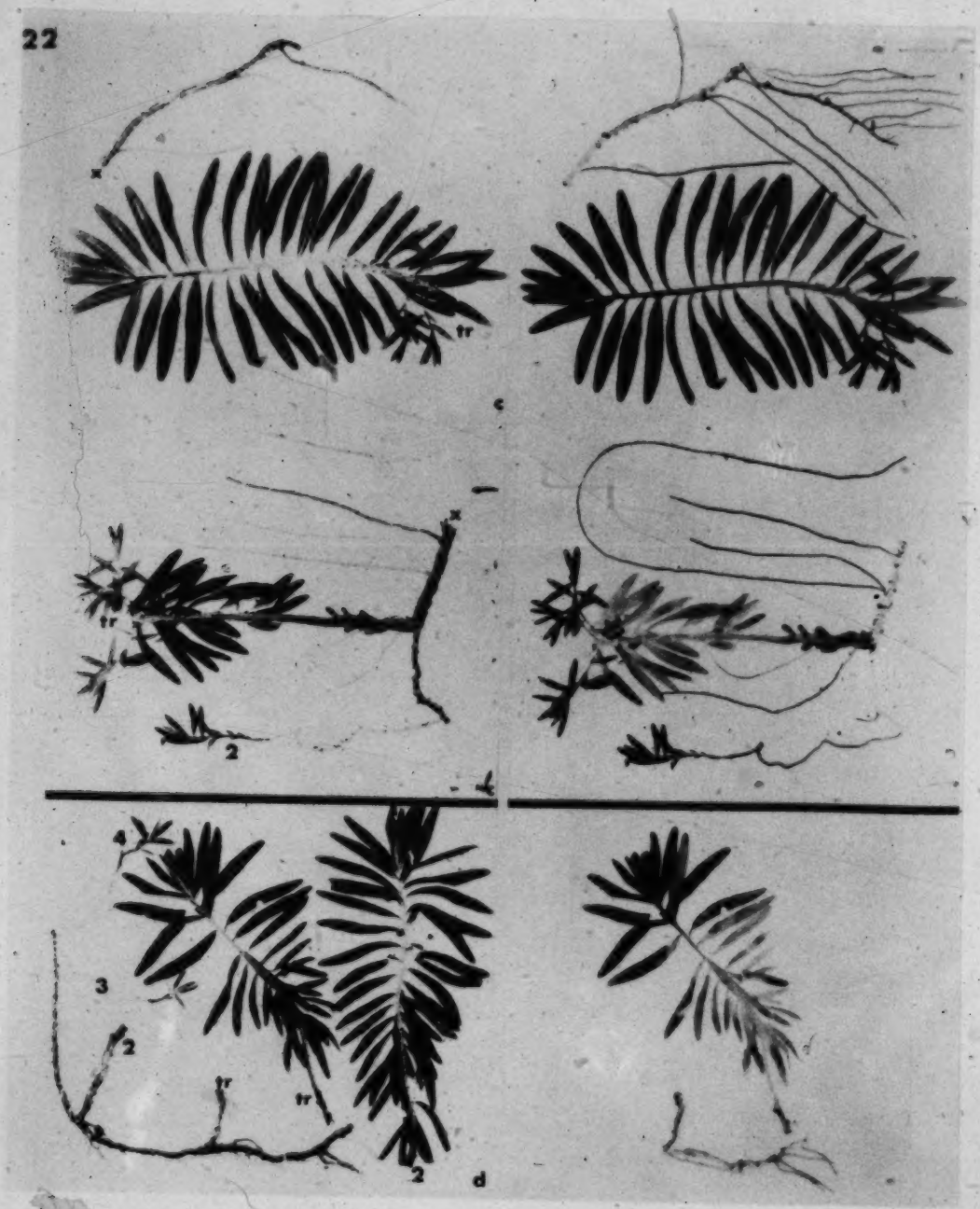
Table 19. Distribution of ^{14}C-assimilates in leafy spurge 48 hours after treatment with $^{14}CO_2$.

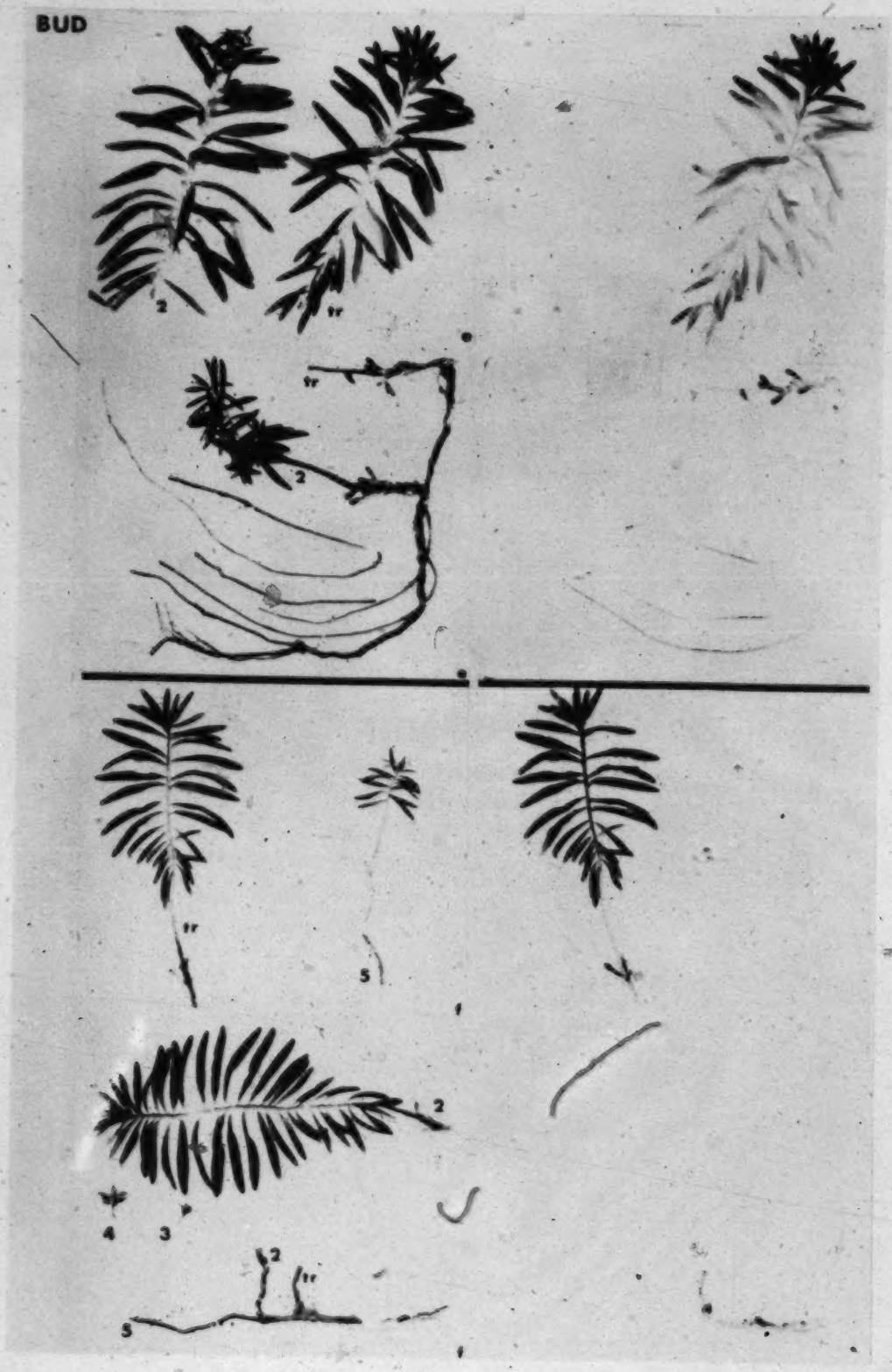
Growth stage of treated shoot; cm	^{14}C recovered, % of total				
	Treated shoot	Shoot no.			Roots
		2	3	4-7	
14	70	0			30
14	32	22			46
22	64	1	2	5	28
22	82	0	0		18
bud (26)	74	3			23
bud (26)	76	0			24

receive any ^{14}C-assimilates from the treated shoot (Fig. 15, plants d, f) could, of course, be importing photosynthates from other large shoots. In a plant with only one large shoot (Fig. 15, plant c) the ^{14}C-assimilates were translocated throughout the entire root system.

Not enough experiments were carried out to draw definite conclusions about glyphosate translocation in leafy spurge. In the experiments done, glyphosate did not bypass any secondary shoots (Table 15, page 70), which suggests that the translocation patterns for assimilates and glyphosate differ.

Figure 15. Distribution of ^{14}C-assimilates in leafy spurge plants 48 hours after $^{14}CO_2$ application to a main shoot (tr.) at three growth stages (14 cm, 22 cm, bud (26 cm)). Left: plant mounts. Right: autoradiograms.


22
c
d
3
2
2


BUD
2
11
11
2
11
5
2
4
3
2
11
5

11.3 Toadflax

Excessive moisture during the month of July reduced the growth rate of toadflax, hence most of the plants available were autoradiographed. The few extracted plants had radioactivity in all secondary shoots. Plant (a) in Fig. 16 produced no secondary shoots but translocated ^{14}C-assimilates throughout its root system. However, translocation of ^{14}C-assimilates may take place to only part of the root system (Fig. 16, plant (c)) suggesting that some younger shoots may be supported by larger secondary shoots, #3 in this case. In plant (b) in Fig. 16, however, a relatively large amount of radioactivity has been translocated to shoot 2, which was the same size as the treated shoot. Translocation to emerging shoots also occurred in plant (b). The larger shoots of plants d and e in Fig. 16 imported little ^{14}C-assimilate, as expected. Smaller shoots imported a greater amount of assimilate. No differences in the translocation of ^{14}C-assimilates were found between plants treated at the three growth stages.

The pattern of assimilate translocation in all three species is comparable. Secondary shoots may import assimilates from the treated main shoot or may be bypassed. The larger of these shoots, however, are probably self-supporting. The reason why they should import assimilates is not clear. Glyphosate may be translocated in the assimilate stream, but differences in translocation patterns between glyphosate and assimilates in both Canada thistle and leafy

Figure 16. Distribution of ^{14}C-assimilates in toadflax plants 48 hours after $^{14}CO_2$ application to a main shoot (tr.) at three growth stages (24 cm, 33 cm, bud (38 cm)). Left: plant mounts. Right: autoradiograms.

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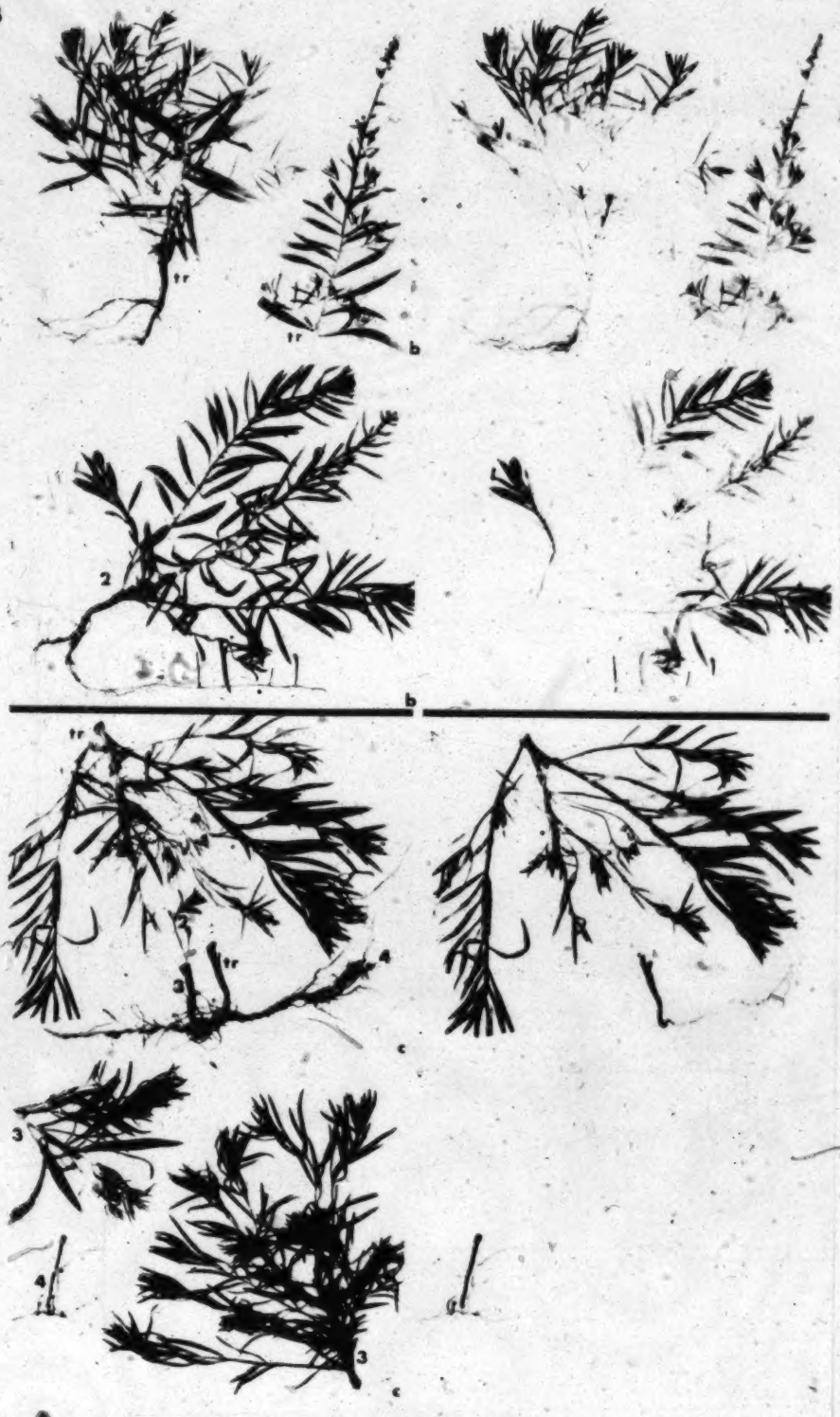
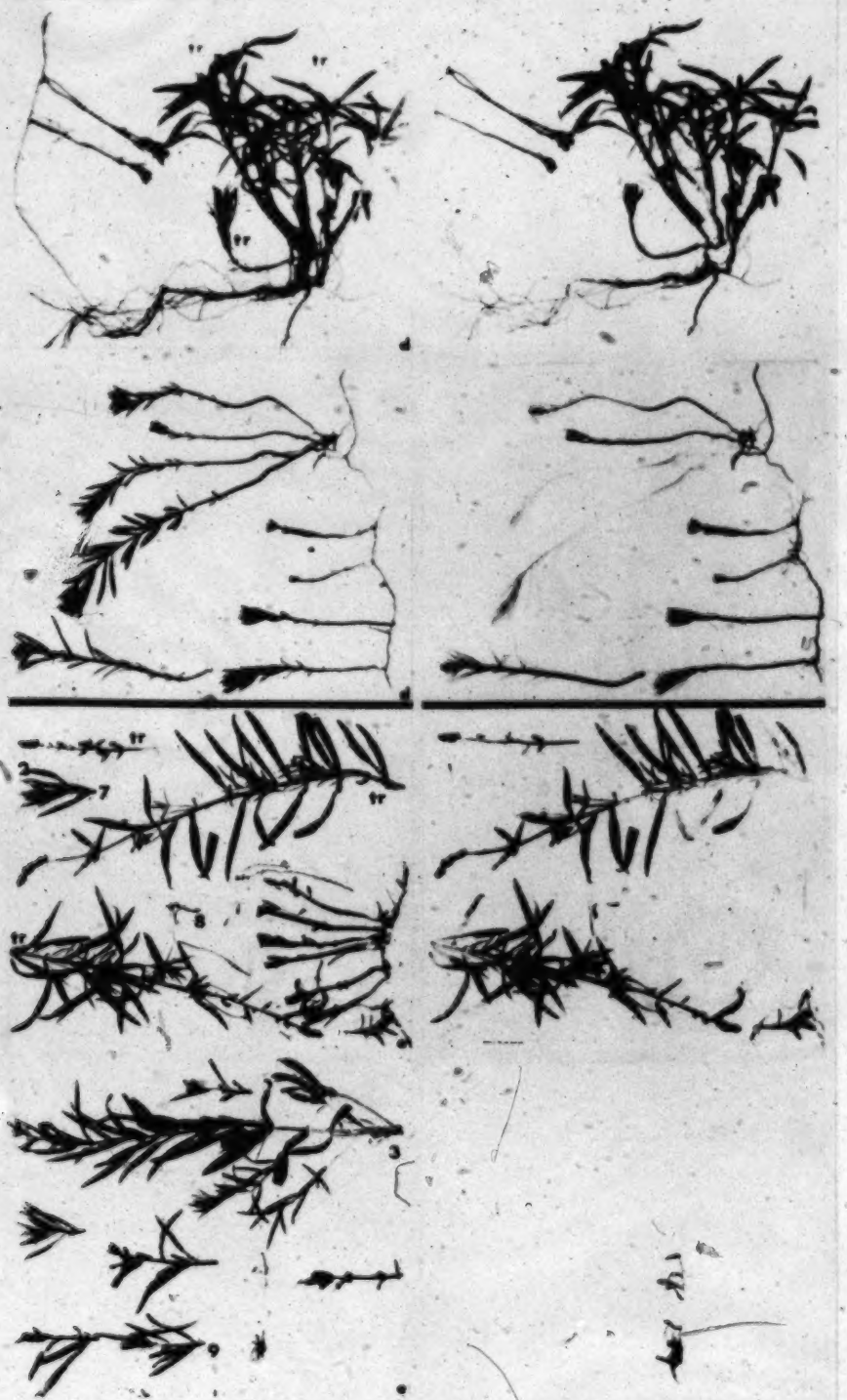


BUD



spurge were observed.

Further investigations are needed for a better understanding of assimilate and herbicide translocation patterns in perennial weeds. Such knowledge is essential since any herbicide, to effectively control perennial weeds, must be translocated to the roots to exert its phytotoxic effects. Further work also is needed to clarify to what extent glyphosate is translocated with the assimilate stream.

SUMMARY AND CONCLUSIONS

Glyphosate effectively controlled Canada thistle plants at all growth stages in the field and in the greenhouse. The most lasting control was obtained after application at a mature growth stage; but application to young regrowth after cutting or harvest was also effective. Control was equally good within the temperature range 7 to 27°C. Leafy spurge was more tolerant to glyphosate than Canada thistle, both in the field (69) and in the greenhouse. The amount of glyphosate taken up after spot treatment of the leaves was low (10 percent) and identical in both species. However, leafy spurge leaves showed poor spray retention which may account for the reduced control. Addition of surfactants to the commercial glyphosate formulation did not improve leafy spurge control.

The glyphosate taken up by Canada thistle was readily translocated to the roots and secondary shoots. This rapid translocation is essential for perennial weed control. Greatly improved uptake and translocation out of the treated leaves of leafy spurge was obtained under high relative humidity. Such increased uptake would be expected to improve control. However, humidity cannot be controlled under field conditions.

In the greenhouse, glyphosate at sublethal doses (0.22 and 0.56 kg/ha) induced changes in the growth of Canada thistle. These changes included production of many new secondary shoots, reduced top growth and swollen roots with a reduced

number of laterals. The xylem in the roots was reduced and diffuse and there was an increased number of xylem fibers. Both xylem and phloem appeared functional.

The amount of ^{14}C-glyphosate present on the leaves decreased with time after application. For the first week (after application) more glyphosate was lost to the environment from the leaf surface than was taken up and translocated out of the leaves. A decrease in the total recoverable radioactivity with time indicates that ^{14}C-glyphosate was metabolized, probably to $^{14}CO_2$ which was subsequently lost to the environment.

Glyphosate was taken up by roots of both Canada thistle and leafy spurge grown in nutrient solution containing the herbicide. It was also readily adsorbed by soil. These results indicate that the lack of glyphosate activity via the soil is not due to lack of root uptake, but rather to soil adsorption and probably microbiological breakdown (46, 66).

Following treatment of a main shoot of Canada thistle, leafy spurge and toadflax with $^{14}CO_2$ in the field, there was a source-to-sink movement of the ^{14}C-assimilates. Photosynthates were translocated to smaller secondary shoots, especially the emerging leaves. Larger secondary shoots, 15 to 20 cm or taller, would in some cases import ^{14}C. If this ^{14}C was imported as ^{14}C-assimilates, this was unexpected since according to Hodgson (38) and Müller (49) 10 cm tall Canada thistle shoots are expected to be exporting photo-

synthates. However, these secondary shoots could have a net export. One possible explanation for the import of ^{14}C could be that the assimilates in the roots were converted to other substances which could then be retranslocated to the secondary shoots. Further work on the fate of the assimilates in perennial weeds is required. Smaller shoots of leafy spurge and toadflax may or may not receive ^{14}C-assimilates. Often young secondary shoots of these two species were self-supporting at an early stage. Shoot buds on the roots were strong sinks for assimilates. A portion of the root system and the shoots emerging from it could be completely bypassed.

Glyphosate did not completely follow the assimilate stream in its translocation pattern. Even if it does follow the assimilate stream in Canada thistle, leafy spurge and toadflax some secondary shoots are still likely to be bypassed. Young shoots that had not emerged at the time of spraying and were bypassed would escape glyphosate treatment and be a source of reinfestation.

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